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THE ANALYST

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THE ANALYST

EDITORIAL

Our New Covers

On our earliest, very Victorian, cover the words "The Analyst" appeared in black Old English type, followed by the contents list and lists of the Committee of Publication and of the abstractors—and there was still room on that front cover for a half-page advertisement for Dr. Muter's books. Later, as the lists of names and titles grew, the cover filled up and, as if to hold all the type in place, it was surrounded by a black band of printer's ornaments a quarter of an inch wide. By the turn of the century, the Editorial Committee found itself driven off the front cover by pressure from the contents list (listing mainly abstracts). They made a come-back within a few years, displacing the Contents to the back page.

The last years of the Edwardian era saw the first really re-designed cover, the words "THE ANALYST" appearing in roman type and the cover being printed in blue on cream papers, colours that persisted until last month. January, 1948, saw the familiar inverted-L shaped rule introduced, a modification that presaged the second real re-designing. The cover of 1950, with its sans-serif type and open spaces, lasted eight years.

A glance at a cover of only eleven years ago discloses an immense clutter of type, 90 per cent. of which has since been removed. The December, 1947, cover contained 150 full words (including proper names), 10 abbreviations of words, 15 numeral groups, 78 groups of letters representing honours or qualifications, and 64 initials, a grand total of 254 items.* This was roughly halved in 1948 by dropping the list of abstractors, a change made imperative by a trebling of their numbers, which would have resulted in an impossible overcrowding of the cover. At the beginning of 1950 the second major re-designing saw the Publication Committee swept on to the back cover. At the same time the abstracts went into *British Abstracts C* for a short period, returning four years later to the Society's fold as a separate (but matching) journal, *Analytical Abstracts*.

Now, once more, we have a new cover, and *Analytical Abstracts* has modified its appearance, although a family resemblance has been retained. Gone from *The Analyst* is the clutter of printed matter—only 40 items of lettering remain. If this new look is reflected in the further simplification of the analyst's task by the discovery of some fundamentally new techniques—and if we are privileged to publish them—we shall be satisfied.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NEW MEMBERS

Ordinary Members

Arthur Charlesworth; Peter George William Cobb, A.R.I.C.; William Crooks Fawcett; Stig K. L. Freyschuss, D.Sc. (Stockholm); Dudley Arthur Harris, M.Sc. (Cape Town), A.R.I.C.; Donald Geoffrey Hayden, M.P.S., D.B.A.; Bryan Seagrave Nichols; Kenneth Ledgard Nicholson; Dennis Price, B.Sc. (Birm.); Philip Alexander Raine, F.R.I.C., C.I.E.E.; Eleftherios C. Stathis, D.Sc. (Athens).

Junior Members

Vincent Paul Lynch; George Anthony Smith.

* If we haven't miscounted.

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NORTH OF ENGLAND SECTION

A JOINT Meeting of the North of England Section and the North Lancashire Section of the Royal Institute of Chemistry was held at 7.30 p.m. on Thursday, December 4th, 1958, at the Storey Institute, Meeting House Lane, Lancaster. The Chair was taken by the Chairman of the North Lancashire Section, Mr. C. D. Lafferty, B.Sc., F.R.I.C.

The following paper was presented and discussed: "Recent Advances in Polarography and Some Other Electrical Methods," by G. F. Reynolds, M.Sc., F.R.I.C.

WESTERN SECTION

A JOINT Meeting of the Western Section with the Cardiff and District Section of the Royal Institute of Chemistry and the South Wales Section of the Society of Chemical Industry was held at 7 p.m. on Friday, December 19th, 1958, at University College, Cardiff. The Chair was taken by the Chairman of the Western Section, Mr. S. Dixon, M.Sc., F.R.I.C.

A lecture on "Recent Trends in Qualitative Analysis" was given by D. W. Wilson, M.Sc., F.R.I.C.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, December 9th, 1958, in the Gas Showrooms, Nottingham. The Chair was taken by the Chairman of the Section, Dr. R. Belcher, F.R.I.C., F.Inst.F.

The following paper was presented and discussed: "The Analysis of Tar Acids," by H. G. Willcock.

AN Ordinary Meeting of the Section was held at 6.30 p.m. on Wednesday, December 17th, 1958, in the Mason Theatre, The University, Edmund Street, Birmingham 3. The Chair was taken by the Chairman of the Section, Dr. R. Belcher, F.R.I.C., F.Inst.F.

A discussion on "Flame Photometry" was opened by L. Brealey, B.Sc.

MICROCHEMISTRY GROUP

THE seventeenth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, December 10th, 1958, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Honorary Secretary of the Group, Mr. D. W. Wilson, M.Sc., F.R.I.C.

A discussion on "The Use of the Microscope in Analysis" was opened by R. L. Causer, B.Sc.

PHYSICAL METHODS GROUP

THE fourteenth Annual General Meeting of the Group was held at 6.30 p.m. on Tuesday, November 25th, 1958, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Mr. R. A. C. Isbell, A.Inst.P. The following appointments were made for the ensuing year:—*Chairman*—Mr. R. A. C. Isbell. *Vice-Chairman*—Mr. G. W. C. Milner. *Hon. Secretary and Treasurer*—Dr. T. L. Parkinson, Product Research Division, Beecham Foods Ltd., Beecham House, Great West Road, Brentford, Middlesex. *Members of Committee*—Messrs. W. Cule Davies, A. L. Glenn, J. H. Glover, C. A. Parker, J. W. Price and G. F. Reynolds. Dr. D. C. Garratt and Mr. C. A. Bassett were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by the 64th Ordinary Meeting of the Group, at which the following papers were presented and discussed: "Preparative Scale Gas Chromatography," by C. R. Patrick, B.Sc., Ph.D., A.R.I.C.; "Some Aspects of the Practice of Zone Refining," by C. R. Veale, Ph.D., A.R.C.S., D.I.C., A.R.I.C. (see summaries below).

PREPARATIVE SCALE GAS CHROMATOGRAPHY

DR. C. R. PATRICK said that in the last few years several successful attempts had been made to apply the principles of gas chromatography to the preparation of large samples of pure materials. Two general methods could be used. In the first, small

samples of the mixture to be resolved were successively injected into a small chromatographic column, and the resulting fractions were accumulated with the aid of automatically operated devices. In the second, a large sample of the mixture was separated on a column of large cross-section.

Dr. Patrick then described the experience gained at the Birmingham University Chemistry Department in the construction and use of large gas-chromatographic columns. He also referred to the published accounts of other large-scale chromatographic apparatus.

Some Aspects of the Practice of Zone Refining

Dr. C. R. Veale said that, when the highest degree of purity was required, physical methods of purification were necessary. Such a method was zone refining, which made use of the differential solubility of impurities in the solid and the liquid matrix. The ideal distribution between solid and liquid could be calculated from the phase diagram, but in practice the efficiency of separation was lower than that predicted.

The various properties (melting-point, volume change on melting, thermal conductivity and so on) of the materials to be purified had led to a considerable variety in technique. Attempts had also been made to overcome the problem of contamination, especially of materials of high melting-point, and to provide for micro, and continuous large-scale, zone refining.

The technique had been applied to metals, metalloids, organic and inorganic compounds and to eutectic mixtures.

BIOLOGICAL METHODS GROUP

The fourteenth Annual General Meeting of the Group was held at 6.30 p.m. on Tuesday, December 9th, 1958, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Dr. S. K. Kon, F.R.I.C. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Dr. J. I. M. Jones. *Vice-Chairman*—Mr. J. S. Simpson. *Hon. Secretary and Treasurer*—Mr. K. L. Smith, Standards Department, Boots Pure Drug Co. Ltd., Nottingham. *Members of Committee*—Messrs. W. A. Broom, S. K. Kon, J. W. Lightbown, R. F. Milton, Magnus Pike and R. E. Weston. Miss J. Stephens was appointed Hon. Recorder and Messrs. D. M. Freeland and J. H. Hamence were re-appointed Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Group, at which an informal discussion was opened by the retiring Chairman, S. K. Kon, Ph.D., D.Sc., F.R.I.C., on "Methodological Reminiscences."

The Determination of Linalol: A Comparative Study of the Glichitch and Fiore Methods by Gas-Liquid Partition Chromatography

By D. Holness

(*Unilever Ltd., Central Perfumery Department, 1, Southwark Park Road, London, S.E.16*)

Work undertaken on behalf of the Essential Oils Sub-Committee of the Analytical Methods Committee

There has been much discussion of the relative merits of the Glichitch (aceto-formic) and Fiore (acetyl chloride - dimethyl aniline) methods for the determination of linalol and other tertiary alcohols in essential oils. Objections to the Glichitch method have been based on the tendency of tertiary alcohols to yield dehydration compounds under conditions of low pH and on the probable formation of acetates as well as formates. Both methods have been stated to give inconsistent results at times.

The problem has received the attention of the Essential Oils Sub-Committee of the Analytical Methods Committee of the Society for Analytical Chemistry, to whom the author suggested the use of gas - liquid chromatography as a means of studying the courses of the two types of reaction. The Sub-Committee asked him to devise and perform a series of experiments to this end.

This paper describes the investigation of the reaction products and discusses their bearing upon the reliability of analytical results. Since the author has himself observed no significant inconsistencies in results between successive determinations, correctly performed, by either method, this aspect of the problem could not be studied by gas - liquid chromatography.

It was found to be undesirable to use natural essential oils in comparative studies, since the presence in them of compounds other than linalol complicates the interpretation of chromatograms. Linalol from rosewood oil was used and certain comparative experiments were made with a synthetic linalol.

The experimental work was divided into four sections, as follows—

(*i*) Samples of linalol were esterified by the Glichitch method; others by the Fiore method. The retention times of the peaks appearing on gas - liquid chromatograms were compared with those of esters of linalol and of other alcohols that can arise by isomerisation of linalol.

(*ii*) Reaction mixtures were saponified, and the retention times of the recovered alcohols were compared with those of linalol and possible isomerides of linalol. The recovered acids were also examined.

(*iii*) Four other peaks observed during the experiments were investigated and shown to derive from impurities present in the natural and synthetic linalols used.

(*iv*) In a series of comparative tests, the alcohol content of a sample of linalol was determined by four analysts, each of whom used both methods. Their reaction mixtures were examined by gas - liquid chromatography.

In all, seventeen different peaks and one other group of peaks due to hydrocarbons required investigation. The nature of compounds originating from linalol was determined with a reasonable degree of certainty. Comparative gas - liquid chromatograms were made under standard conditions, so that retention times could be taken as proportional to retention volumes. The column used was 180 cm in length. It was packed with Celite, with "polypropylene sebacate" as stationary phase. The standard column temperature was 135° to 136° C and the rate of flow of the carrier gas (nitrogen) was 40 ml per minute.

Throughout the investigation, the two analytical methods were performed as described previously.[1]

Experimental

Figs. 1 and 2 are gas - liquid chromatograms of rosewood oil and ho-leaf oil after treatment by the Glichitch method; Figs. 3 and 4 are chromatograms of the same oils after treatment by Fiore's method. The group of peaks numbered (1) is caused, in the essential oils before esterification, by naturally occurring terpenes, but similar positions are occupied by unsaturated hydrocarbons resulting from the loss of a molecule of water from linalol or of a molecule of acid from any of its esters. It was chiefly because of the need to study the extent of this type of breakdown, which leads to low analytical results, that terpene-free linalol was chosen for further work; but also because other alcohols, which can give rise to ester peaks, such as those numbered (13) and (15) in Fig. 3, are present in natural oils.

Compounds derived from linalol—

Linalol from rosewood oil, treated by the Glichitch method, gave the chromatogram shown in Fig. 5. Treated by Fiore's method, it gave the chromatogram shown in Fig. 6. The main peak in Fig. 5 corresponds to linalyl formate and that in Fig. 6 to linalyl acetate. Group (1) peaks, which are produced by isomeric linalol anhydrides, are more pronounced in Fig. 5. A small amount of unesterified linalol—peak (4)—appears on each chromatogram. Additionally, Fig. 6 shows evidence of at least one other component and Fig. 5 of at least eight. The half-peak (6) in Fig. 5 corresponds in position to linalyl acetate; the others, peaks (7) and (9) to (15), remain to be identified. It is clear that the Glichitch peaks must

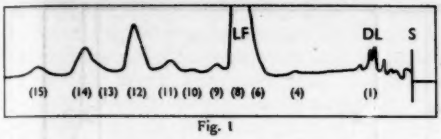

Fig. 1

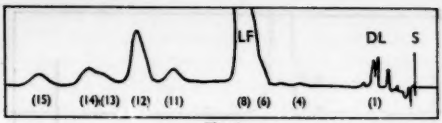

Fig. 2

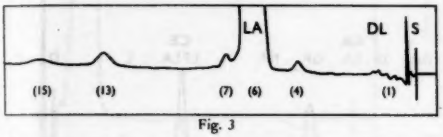

Fig. 3

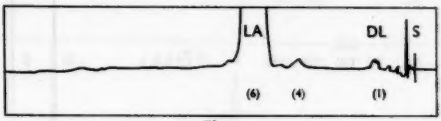

Fig. 4

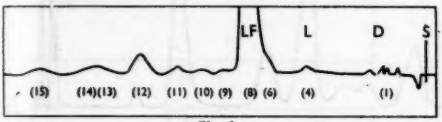

Fig. 5

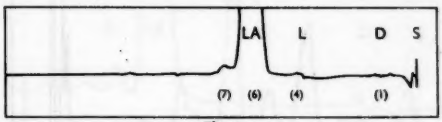

Fig. 6

Fig. 1. Rosewood oil esterified by Glichitch method
Fig. 2. Ho-leaf oil esterified by Glichitch method
Fig. 3. Rosewood oil esterified by Fiore method
Fig. 4. Ho-leaf oil esterified by Fiore method
Fig. 5. Rosewood linalol esterified by Glichitch method
Fig. 6. Rosewood linalol esterified by Fiore method

In Figs. 1 to 6, D = linalol anhydrides, DL = natural terpenes and linalol anhydrides, L = linalol, LA = linalyl acetate, LF = linalyl formate, S = start

be caused mainly by esters because of the high ester value of the formylated oil, which is equivalent to about 96 per cent. of linalyl formate.

It is known that linalol can undergo rearrangement to yield the *cis* and *trans* isomers, geraniol and nerol. Ring closure can also occur, with the formation of at least one of the isomeric terpineols. Geraniol, nerol and terpineol have been isolated from the products of esterification of linalol under acid conditions at temperatures of 100° to 120° C. They would certainly be present if a Glichitch-type reaction were performed at higher temperatures. Accordingly, Glichitch reaction mixtures from rosewood linalol and synthetic linalol were heated for a short period in a boiling water-bath, when the chromatograms shown in Figs. 7 and 8 resulted.

Ignoring for the moment the absence of peaks (9) and (10) from formylated synthetic linalol, it is noticeable that there is no difference in the numbers or positions of the peaks whether the Glichitch test is performed hot or cold. Heating appears only to increase the proportions of linalol anhydrides and other compounds at the expense of linalyl esters. The unknown Glichitch peaks were therefore compared with the formates and acetates of geraniol, nerol and terpineol. When the respective retention times were measured, it was found that peak (11) corresponds to neryl formate, peak (12) to geranyl formate, peak (13) to neryl acetate and a terpinyl acetate, peak (14) to a terpinyl formate and peak (15) to geranyl acetate. Although such agreement is significant, it cannot be taken to constitute proof of identity, so further confirmation was sought. The ester mixtures were saponified and the acids were separated. The remaining oils gave the gas - liquid chromatograms shown in Figs. 9 and 10. Peak (4) corresponds to linalol, and the retention times of peaks (G), (N) and (T) are the same as those of geraniol, nerol and one of the terpineol isomers. Further, the relative amounts of alcohols indicated are much as might be expected from an ester mixture of the type postulated. The same peaks were shown by saponified standard Glichitch reaction mixtures, although in these linalol predominated.

Examination of the recovered acids from the recommended Glichitch method reaction mixture revealed the presence of formic and acetic acids in the approximate ratio of 2 to 1. Peaks (11) to (15), therefore, must definitely include both formates and acetates of alcohols other than linalol and are probably due to the compounds previously mentioned. It is also clear from Figs. 6, 16, 18, 20 and 22 that these compounds do not occur in detectable amounts in the products of the Fiore reaction.

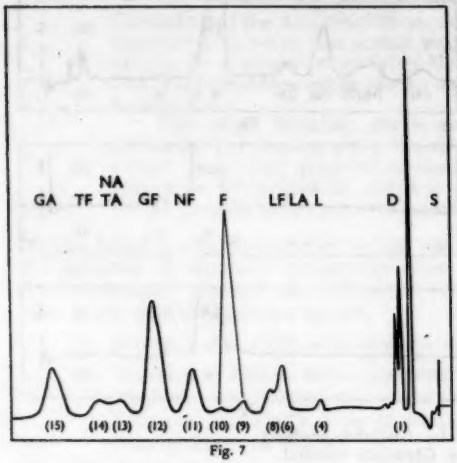

Fig. 7

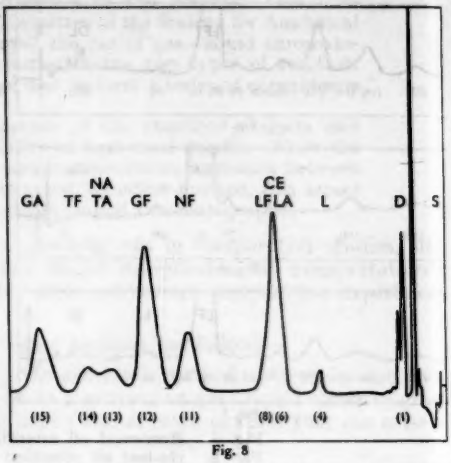

Fig. 8

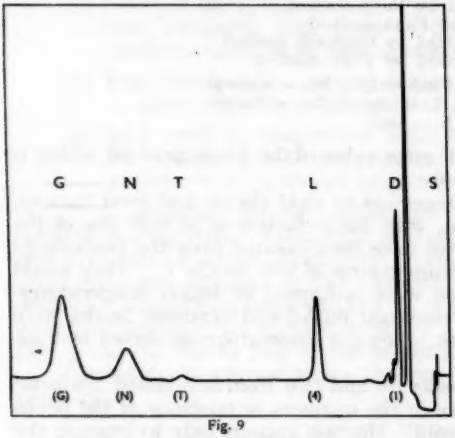

Fig. 9

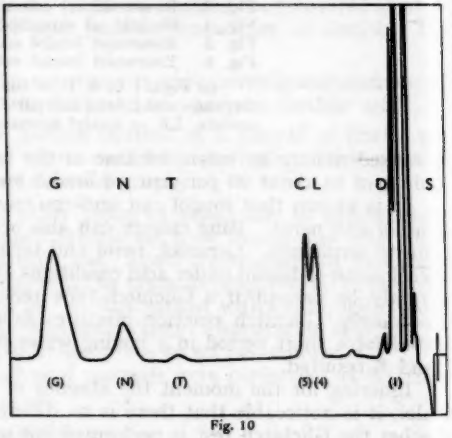

Fig. 10

Fig. 7. Rosewood linalol esterified by Glichitch method and heated to 100° C
Fig. 8. Synthetic linalol esterified by Glichitch method and heated to 100° C
Fig. 9. Rosewood linalol esterified by Glichitch method; after saponification
Fig. 10. Synthetic linalol esterified by Glichitch method; after saponification

In Figs. 7 to 10, in addition to symbols used in Figs. 1 to 6, C = alcohol C, CE = alcohol C esters, F = A and B formates, G = geraniol, GA = geranyl acetate, GF = geranyl formate, N = nerol, NA = neryl acetate, NF = neryl formate, T = terpineol, TA = terpinyl acetate, TF = terpinyl formate

COMPOUNDS NOT DERIVED FROM LINALOL—

It remained to account for the origins of the peaks numbered (7) in Fig. 6 and (9) and (10) in Fig. 5; also peak (5), which appeared after saponification of acetoformylated synthetic linalol (Fig. 10).

Peak (5) was shown to correspond with an impurity (designated "Alcohol C"—Fig. 11) present originally in the synthetic linalol. It is thought to be an alcohol, the formate and acetate of which cannot be differentiated from those of linalol on chromatograms made under the conditions used. Alcohol C could not be found in linalol from rosewood oil.

Peaks (7), (9) and (10) are observed only on chromatograms of rosewood oil (see Figs. 1 and 3) and linalol prepared from rosewood (see Figs. 5, 6 and others). They are absent

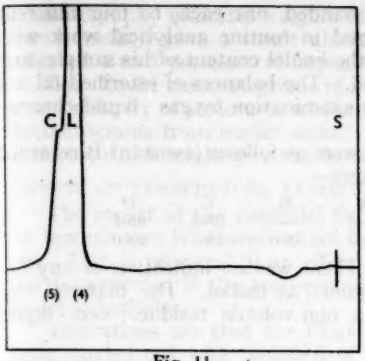

Fig. 11

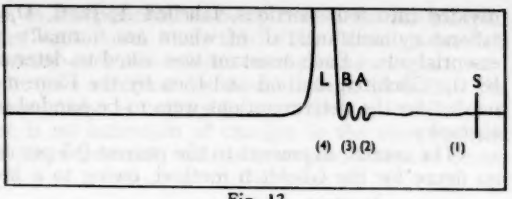

Fig. 12

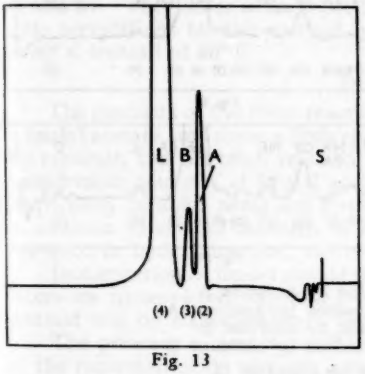

Fig. 13

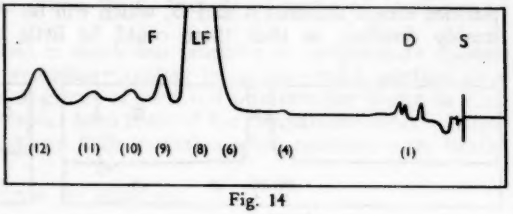

Fig. 14

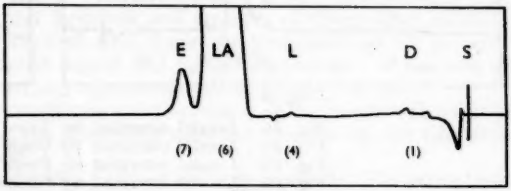

Fig. 15

Fig. 11. Impurity in synthetic linalol
Fig. 12. Impurities in rosewood linalol
Fig. 13. Impurities in head fractions of rosewood linalol
Fig. 14. Head fractions of rosewood linalol esterified by Glichitch method
Fig. 15. Head fractions of rosewood linalol esterified by Fiore method

In Figs. 11 to 15, in addition to symbols used in Figs. 1 to 10, A = alcohol A, B = alcohol B, E = acetate of "heads" alcohol A or B

when ho-leaf oil (see Figs. 2 and 4) and synthetic linalol (see Figs. 8 and 25) are esterified. Their source was sought in impurities in linalol from rosewood; impurities originating in the rosewood oil from which it had been distilled. This linalol from rosewood gave a chromatogram on which two small peaks, numbered (2) and (3), preceded that of linalol itself (see Fig. 12). Fractional distillation yielded heads in which the two peaks were augmented (see Fig. 13). These heads were esterified by the Fiore method (see Fig. 15) and by the Glichitch method (see Fig. 14). In the first instance, the size of peak (7) was clearly greater (compare Figs. 6 and 15); in the second, peaks (9) and (10) were increased (compare Figs. 5 and 14). Peaks (2) and (3) were consequently attributed to two unidentified alcohols from rosewood oil (designated "A" and "B"), the formates of which give rise to peaks (9) and (10). One of them apparently forms an acetate responsible for peak (7); the acetate of the other is presumed to be hidden within the large linalyl acetate peak.

It was not considered important, for the purpose of this investigation, to attempt to identify the supposed alcohols A, B and C.

COMPARATIVE TESTS—

Once the origins of the various peaks were understood, a series of comparison tests was performed, both analytical methods being used. A sample of linalol from rosewood was

divided into four portions, labelled A, B, C, D, and handed, one each, to four different laboratory assistants, all of whom are normally engaged in routine analytical work with essential oils. Each assistant was asked to determine the linalol content of his sample, first by the Glichitch method and then by the Fiore method. The balances of esterified oil not needed for the determinations were to be handed in for examination by gas - liquid chromatography.

The results, expressed to the nearest 0·5 per cent., were as follows (assistant B returned no figure for the Glichitch method, owing to a breakage)—

Assistant	A	B	C	D
Linalol found by Glichitch method, %	95·0	—	95·5	95·5
Linalol found by Fiore method, %	99·0	99·0	98·5	98·0

Fig. 12 shows all the peaks of the linalol used. There was no indication of any impurities except alcohols A and B, which will be determined as linalol. The material was freshly distilled, so that there could be little or no non-volatile residue. Gas - liquid

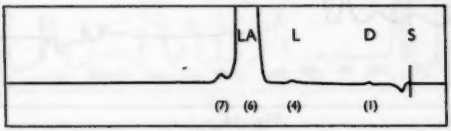

Fig. 16

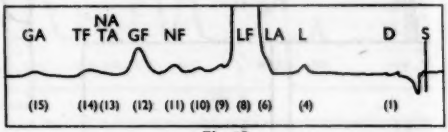

Fig. 17

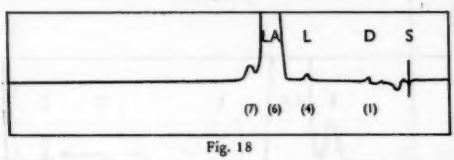

Fig. 18

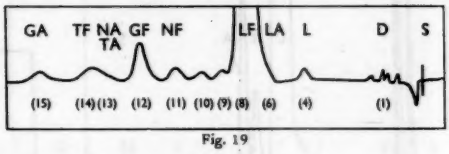

Fig. 19

Fig. 16. Linalol esterified by Fiore method by assistant A
Fig. 17. Linalol esterified by Glichitch method by assistant A
Fig. 18. Linalol esterified by Fiore method by assistant B
Fig. 19. Linalol esterified by Glichitch method by assistant B

In Figs. 16 to 19, symbols used are as for Figs.1 to 15

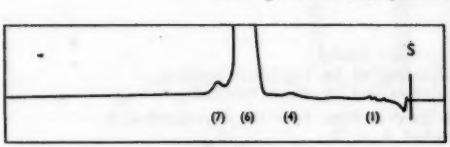

Fig. 20

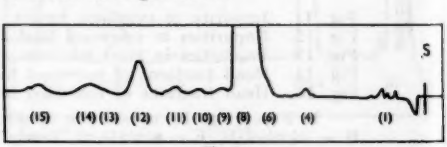

Fig. 21

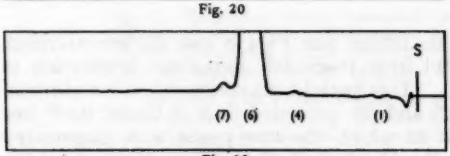

Fig. 22

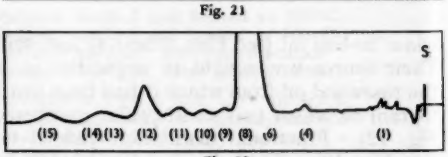

Fig. 23

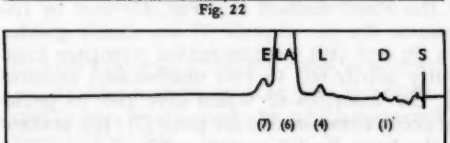

Fig. 24

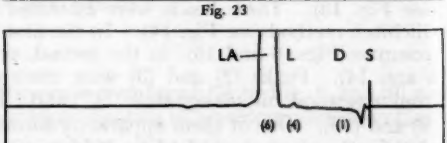

Fig. 25

Fig. 20. Linalol esterified by Fiore method by assistant C
Fig. 21. Linalol esterified by Glichitch method by assistant C
Fig. 22. Linalol esterified by Fiore method by assistant D
Fig. 23. Linalol esterified by Glichitch method by assistant D
Fig. 24. Rosewood linalol esterified by Fiore method without cooling
Fig. 25. Synthetic linalol esterified by Fiore method without cooling

chromatography indicates a figure of close to 100 per cent. as being correct for this sample. Fiore's method gave a result about 1 per cent. lower than this. The Glichitch method indicated 4 to 5 per cent. of impurities, which were not evident on the chromatogram.

Gas - liquid chromatograms of all eight ester mixtures are shown in Figs. 16 to 23. The great similarity of structures within the two groups is noticeable, as is their resemblance to chromatograms from earlier tests. There is no indication of changes in the course of the reactions, which might give rise to serious inconsistencies, such as have been stated to occur.

Effects of temperature variation—

The report of the Essential Oils Sub-Committee[1] says of the Glichitch method that, if the temperature is allowed to fall below 20° C during the standing period, results may be low. This is presumably an effect caused by incomplete esterification. Low results may also be expected if the temperature rises above 22° C, because of increased formation of linalol anhydrides.

Indications are that the Fiore method is much less sensitive to temperature change. Tests carried out with omission of the preliminary cooling in an ice - water mixture gave results of 98 to 99 per cent. Two chromatograms of such test mixtures are shown in Figs. 24 and 25. They differ in no important respect from those of the comparative tests. Large-scale acetylations by this method have shown little variation when mixtures were heated at 80° C instead of 40° C.

Discussion of results

The products of the Fiore reaction with linalol are much the simpler. The chief product is linalyl acetate, and there is little else except traces of unreacted linaol and linalol anhydrides. By contrast, the Glichitch reaction is complex. Linalyl formate is the main product, but considerable amounts of linalyl acetate and formates and acetates of three other alcohols (apparently geraniol, nerol and terpineol derived from linalol by isomerisation) are present, in addition to smaller amounts of unreacted linalol and linalol anhydrides. When oils of rosewood or ho-leaf are used, the two reactions apparently follow similar courses.

Isomerisation of linalol should not in itself affect the accuracy of the result. If terpinyl esters are incompletely saponified during the ester determination, results by the Glichitch method will be correspondingly decreased.

The presence of acetates will reduce results by the Glichitch method. The equivalent of the recovered acids suggests a molar proportion of formic to acetic acids of 3 to 1, *i.e.*, 25 per cent. of acetates. This would decrease the result by a little less than 2 per cent. Peak resolution on the chromatograms is not good enough to permit accurate measurement, but it indicates a somewhat smaller proportion of acetates, not exceeding 15 per cent., which is sufficient to decrease the result by 1 per cent.

Incomplete esterification was observed in both methods. On Fiore chromatograms, the free linalol content is always below an apparent 1 per cent., whereas Glichitch chromatograms indicate a somewhat higher average (1 per cent. or more, but less than 2 per cent.).

Linalol anhydrides are formed only in traces during the Fiore reaction. The total content seems always to be well below 1 per cent. The Glichitch reaction gives more variable amounts, generally of the order of 1 per cent., but not more than 2 per cent.

When the possible sources of error revealed by gas - liquid chromatograms are added together, there are good grounds for expecting results by the Fiore method to be some 1 per cent. lower and those by the Glichitch method to be some 3 to 5 per cent. lower than the true linalol content. This agrees well with the observed results in the comparative tests.

The errors referred to are distinct from and additional to the normal types of manipulative error, which, in my experience, may easily amount to ± 1 per cent. in either test.

Conclusions

(*i*) The reaction products of analysis by the Glichitch method are far more complex than those of analysis by Fiore's method. Inconsistencies, on the whole, are less likely to be encountered in simpler reactions.

(*ii*) Nevertheless, each method seems able to give not only repeatable results, but also consistent patterns on gas - liquid chromatograms.

(*iii*) No major sources of error have been found in either method.

(*iv*) Because of the greater complexity of the reaction, there are more potential sources of small errors in analysis by the Glichitch method.

(*v*) Results by Fiore's method will normally be about 1 per cent. lower than the true amount of linalol present; results by the Glichitch method will normally be about 4 per cent. low.

(*vi*) Fiore's method is less sensitive to temperature variations than the Glichitch method is reported to be.

(*vii*) The preference of the Essential Oils Sub-Committee for the Glichitch method, "on account of its greater dependability," needs reviewing in the light of indications that its results, even although they are reproducible, are consistently incorrect.

I thank Messrs. Unilever Ltd. for permission to publish these results.

REFERENCE

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Received *September 15th*, 1958

Volumetric Analysis of Stannous and Total Tin in Acid-soluble Tin Compounds*

BY JOHN D. DONALDSON AND WOLFGANG MOSER

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The procedures and the apparatus described permit accurate determination of stannous and total tin in acid-soluble tin compounds. Results are referred to a recognised primary standard (arsenious oxide), and all standard solutions used are stable indefinitely. Oxidation of stannous tin, whether by atmospheric oxygen or oxygen dissolved in the titrant, is prevented by apparatus design, without recourse to empirical standards, such as tin metal, or empirical additives, such as titanous chloride.

IN the analysis of tin compounds, most workers report the metal in only one oxidation state, and determine it either by complete reduction to stannous tin or complete oxidation to stannic tin. Most volumetric procedures, moreover, are referred empirically to metallic tin. The aim of the proposed procedure is to determine both stannous and total tin by essentially the same volumetric procedure and to refer this procedure to a recognised primary standard.

As has been known for a considerable time, results obtained by the direct titration of stannous solutions with oxidising agents are inaccurate.[1] Errors caused by atmospheric oxidation can be overcome by working in an oxygen-free atmosphere, but those caused by induced oxidation by oxygen dissolved in the titrant cannot. These difficulties can be partly overcome by standardisation against pure tin, when all oxidation errors are assumed to be constant; Evans,[2] however, pointed out the unsatisfactory nature of such procedures. Okell and Lumsden[3] evolved a satisfactory direct titration with air-free iodine that had been standardised against arsenious oxide; the disadvantages of the method are that standard iodine solutions do not maintain a constant normality if stored for any length of time and that the titrations are carried out in the presence of air, so that errors caused by atmospheric oxidation of stannous tin during titration are not excluded.

In other methods,[4,5] direct titration of stannous tin has been avoided by oxidising the tin with an excess of oxidant and either back-titrating or determining the equivalent of reducing agent formed. None of these methods, however, has entirely overcome the effect of oxygen dissolved in the oxidant.

The determination of total tin by similar volumetric methods depends on the complete reduction of the tin, usually by means of metallic reductors. Many metals[6] and amalgams have been used for this purpose, including iron, manganese, nickel, cobalt, zinc, aluminium,[7] antimony and lead. Yoshimura[8] showed that the presence of nitrate in determinations of this type leads to inaccurate results caused by interference of nitrite, which he overcame by adding urea.

* Presented at the meeting of the Society on Wednesday, November 5th, 1958. (Also presented at the meeting of the Scottish Section on Wednesday, October 15th, 1958.)

Wood and Clark's[9] recent method for determining tin in zirconium alloys depends on the presence of empirical amounts of titanous ion to counteract the effect of dissolved oxygen, these amounts being approximately valid for samples containing up to 20 per cent. of tin. The authors admit that higher percentages would require a fresh empirical assessment.

Experimental

The proposed method consists of the injection of an excess of freshly boiled ferric chloride solution into an acid stannous solution in a non-oxidising atmosphere, followed by cooling and titration of the resulting ferrous iron with standard ceric sulphate solution. Induced oxidation of ferrous ions by oxygen dissolved in the ceric solution is very slow in the cold and can be neglected.

The production of small amounts of ferrous iron in solutions of ferric chloride by boiling[10] and by prolonged exposure to light[11] has been reported. The amount of reduction caused by boiling is minute, and is negligible for the procedure described. Storage of the ferric chloride solution, in diffuse daylight for 1 month, produced no detectable ferrous iron. For prolonged storage, however, we recommend that the ferric chloride solution be kept in the dark.

A considerable amount of work has been done by the authors with standard potassium dichromate as titrant, but indicator difficulties reduced the over-all accuracy of the method. Ceric sulphate is therefore preferred, although it requires standardisation against a primary standard. We have little doubt that, with the eventual introduction of a completely stable and reversible indicator of $E_0 \simeq -0{\cdot}9$ volt, potassium dichromate would be more convenient.

For determining stannous tin, the sample solution is used directly, but, for total tin, all stannic material is first reduced with aluminium. This metal has been chosen because any excess dissolves completely in the acid used, and cannot therefore affect subsequent stages of the determination. Attempts to remove the excess of a metallic reductor before injection of the ferric chloride solution would lead to unnecessary complication of the apparatus or to the introduction of oxygen. Any metal remaining undissolved would reduce the ferric iron injected, thus leading to high tin assays. Even platinum and silver can reduce strongly acid ferric chloride. Some authors[12] have objected to the use of aluminium because it deposits metallic tin or because splashing may occur during the reaction. In practice, any tin deposited is very spongy and can rapidly be re-dissolved in concentrated hydrochloric acid, while errors from splashing can be avoided both by apparatus design and control of the acid concentration. According to Smith,[13] the efficiency of the reaction depends on the acid concentration, reduction being incomplete if the reaction is either too rapid or too slow.

Method

Apparatus—

The apparatus used is shown in Fig. 1; it consists of a 250-ml reaction vessel, A, connected, through tap B, to a Tucker carbon dioxide generator and, through tap C, to a 100-ml pear-shaped boiler, D, which can be opened to the atmosphere through tap F. A mercury valve, E, consisting of a 3-mm layer of mercury above a No. 2 sintered-glass disc, is fitted to the top of the reaction vessel to release any excess pressure that may arise during analysis. A constriction, G, prevents the mercury from splashing. All solutions are first boiled in flask D and then driven into flask A by their own vapour pressures. The apparatus is assembled by means of B24 ground-glass joints on the reaction vessel and the boiler, by a B14 joint between the mercury trap and the reaction vessel and by rubber tubing on the connecting tubes.

Reagents—

Ceric sulphate solution, 0·1 N—Add 84 g of technical grade ceric oxide in small amounts at a time to a mixture of 160 ml of concentrated sulphuric acid and 120 ml of water, keeping the temperature between 95° and 100° C. Cool, dilute and filter the mixture, dilute the filtrate to 2 litres and standardise[14] it against freshly re-sublimed arsenious oxide.

1:10-*Phenanthroline - ferrous sulphate indicator solution*, 0·025 M.

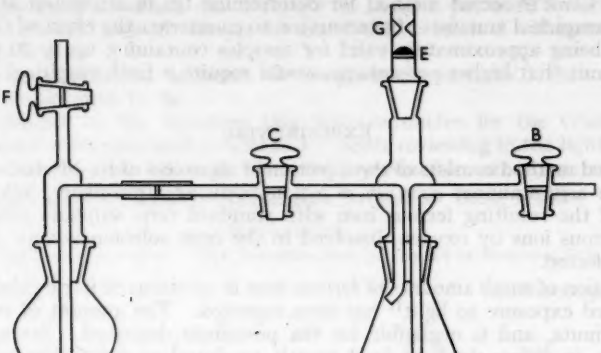

Fig. 1. Apparatus for the determination of stannous and total tin

Ferric chloride solution—Prepare a solution containing approximately 108 g of analytical-reagent grade hydrated ferric chloride, $FeCl_3.6H_2O$, and 178 ml of concentrated hydrochloric acid in 2 litres.

Orthophosphoric acid, sp.gr. 1·75.

Aluminium—British Chemical Standard (containing 99·99 per cent. of aluminium), obtained from the Bureau of Analysed Samples.

PROCEDURE FOR STANNOUS TIN—

Place a weighed sample containing about 0·15 g of stannous tin in the reaction vessel, A, and, with tap C closed, sweep out this vessel with carbon dioxide. Place 50 ml of 2 *N* hydrochloric acid in the boiler, D. De-aerate the acid and inject it into the reaction vessel in the following way—

(*i*) Open taps C and F, and allow carbon dioxide to bubble through the liquid for 5 minutes.

(*ii*) Close tap C and boil the liquid for 2 minutes.

(*iii*) Close tap B, open tap C, close tap F and heat the boiler gently to inject the acid into the reaction vessel.

Close tap C, open taps F and B, and heat the reaction vessel on a water bath until the sample has completely dissolved.

Place 50 ml of ferric chloride solution in the boiler, D. De-aerate the solution and inject it into the stannous solution in the reaction vessel by repeating steps (*i*), (*ii*) and (*iii*). Close tap C, open taps F and B, and heat the reaction vessel for about 10 minutes on a water bath. Cool the reaction vessel, which is still under pressure of carbon dioxide, then dismantle the apparatus and wash any liquid in the connecting tubes into the reaction vessel with distilled water. Add sufficient orthophosphoric acid to decolorise the ferric chloride complex, and titrate the solution with 0·1 *N* ceric sulphate in presence of 1 : 10-phenanthroline - ferrous sulphate solution as indicator.

PROCEDURE FOR TOTAL TIN—

Place a weighed sample containing about 0·15 g of tin in the reaction vessel, dissolve it in 2 *N* hydrochloric acid and add sufficient concentrated hydrochloric acid to make the solution clear. (If necessary, bring the concentration of free acid below 4 *N* by adding solid sodium hydrogen carbonate.) Add about 0·2 g of chemically pure aluminium, connect up the apparatus, and allow carbon dioxide to bubble through. After about 30 minutes, heat the reaction vessel to dissolve the excess of aluminium. (The apparatus is so designed that any splashings caused by hydrogen evolution are washed back into the vessel by the steam evolved on heating.) Inject 30 to 40 ml of concentrated hydrochloric acid to dissolve the deposited tin, and determine the total tin as stannous tin by the procedure described previously.

Results

The accuracy of the proposed procedure for stannous tin was tested in a series of determinations on chemically pure tin (containing 99·98 per cent. of tin), concentrated hydrochloric acid being injected in place of the 2 *N* hydrochloric acid to dissolve the sample; the results were as follows—

Weight of tin taken, g ..	0·1417	0·1464	0·1486	0·1504	0·1765
Weight of tin found, g ..	0·1416	0·1463	0·1485	0·1502	0·1767

Observational errors[15] were calculated for the weights of tin taken and found. The results agreed to within the limits of the calculated observational errors, which account for deviations of ± 0·3 mg for all weights of tin shown.

The accuracy of the proposed procedure for total tin was tested by dissolving weighed amounts of chemically pure tin in concentrated hydrochloric acid, oxidising the solutions with analytical-reagent grade hydrogen peroxide, removing the excess of hydrogen peroxide by boiling and determining the amounts of total tin present in the manner described. The results, which again agree to within the limits of calculated observational error, were as follows—

Weight of tin taken, g ..	0·1187	0·1206	0·1276	0·1304	0·1452	0·1452	0·1465
Weight of tin found, g ..	0·1188	0·1203	0·1275	0·1306	0·1455	0·1452	0·1468

The reproducibility of the proposed method was found by means of a series of forty determinations on typical tin compounds, the results of which are shown in Table I. The observed spread of results is compared with that calculated from the observational errors in weighing the samples and carrying out the titrations.

Table I

Reproducibility of results

Compound	Amount of stannous tin, %	Spread of results, %	Amount of total tin, %	Spread of results, %	Calculated spread,[15] %
Blue-black stannous oxide ..	87·6 87·5 87·5 87·4 87·3	0·3	88·0 87·7 88·0 88·1	0·4	0·4
Stannous sulphate (old sample)	51·3 51·0 51·0 51·0 51·1	0·3	n.d.	n.d.	0·3
Basic stannous nitrate* ..	64·4 64·2 64·1 64·4 64·0	0·4	65·9 65·7 65·7 65·7	0·2	0·4
Stannous hydroxide	79·2 79·0 79·0 79·3 79·1	0·3	79·3 79·5 79·4 79·2	0·3	0·4
Ammonium chlorostannate ..	—	—	33·0 33·1 33·1 33·0	0·1	0·2
Sodium stannate	—	—	37·8 37·6 38·0 38·0	0·4	0·3

* Work on the formulation and chemistry of the basic nitrate is at present in progress, and will be reported elsewhere.

The standard deviation was calculated from all the results in Table I by the formula[16]—

$$\text{s.d.} = \sqrt{\frac{\Sigma d_1^2 + \Sigma d_2^2 + \text{- - - -} \Sigma d_k^2}{N - k}}$$

where N is the number of observations, k is the number of samples and Σd_1^2 - - - etc. is the sum of the squares of the deviations from the mean for samples 1 - - - k. The results were calculated to two decimal places for the purpose of the calculation and the standard deviation was found to be 0·12 per cent., *i.e.*, in 19 cases out of 20, the error should not exceed 0·24 per cent.

We are grateful to Dr. R. A. Chalmers for helpful discussion and to the D.S.I.R. for a maintenance grant to J.D.D.

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Discussion

Mr. F. L. Okell, who opened the discussion at the invitation of the President, congratulated the authors on the ingenuity they had shown in devising a piece of apparatus and a method of titration that effectively overcame the errors to which volumetric tin assays were liable by oxygen dissolved in the standard titrant and by adventitous atmospheric oxidation during the course of the titration. We now had a completely independent reference method for determining tin.

The method was limited in its scope, as was indicated by the title of the paper, but that did not detract from its value; because there was no general method for the determination of tin that was applicable in all circumstances.

Ferric chloride was not new in tin assaying; it was in use for the direct titration of stannous chloride at the Staatshütten laboratory, Essen Ruhr, at least 50 years ago. The reaction took place readily in the cold; this made it difficult to understand why the authors found it necessary to heat the reaction vessel after injecting the ferric salt solution.

No mention of interfering elements was made by the authors, but it might be worth pointing out that ferric chloride was inadmissible in presence of titanium, because it reacted stoicheiometrically with stannous chloride; also that aluminium reduction could not be used in solutions containing antimony, because it reduced antimony to the metal, which then reduced the ferric to ferrous chloride.

He said it might be useful if the paper included information on the form of the aluminium used for reduction. Foil, turnings, ribbon and powder had all been used. The various forms sometimes showed differences in their rate of reaction.

Efficient cooling under pure carbon dioxide was essential; if this gas was prepared from marble and hydrochloric acid, it should be passed through zinc turnings or a few feet of lead pipe to remove any traces of free chlorine, a common impurity in the acid.

In his opinion, too much was made of atmospheric oxidation during the usual iodine or iodate titration. If the neck of a round "boiling flask," full of carbon dioxide, was held close up to the jet of the burette, the titration could easily be finished in less time than that taken by atmospheric oxygen to diffuse through the layer of gas and become dissolved in the assay solution. Any doubts as to the efficacy of this method of conducting the titration, or of titanous chloride in preventing extraneous oxidation, could now be resolved by testing those methods against that of Donaldson and Moser.

Mr. Donaldson replied that the method had been developed mainly because none of the existing methods of tin analysis could be used satisfactorily to determine both stannous and stannic tin in pure tin compounds of widely varying tin content. In the usual iodine or iodate method it was customary to titrate

the tin directly in open flasks, at most maintaining a blanket of carbon dioxide over the solution. The accuracy of the method then depended on the ability of the operator to obtain reproducible errors in a rapid titration. The authors did not therefore believe that they had overstressed the significance of atmospheric oxidation in tin determinations.

It had been pointed out that there was no need to heat the reaction vessel after the injection of the ferric chloride solution. This was perfectly correct. The vessel was, however, standing on a water bath before the injection and there seemed to be no particular reason for removing it.

Aluminium turnings were, in fact, used for the reduction of the stannic tin; the actual rate of reduction in the acid solutions used was not of great importance because any stannic material remaining was finally reduced during the dissolution of the deposited tin metal.

The rate of flow of carbon dioxide from the Tucker generator was checked by bubbling the gas through a solution of potassium hydrogen carbonate. The possibility, slight in any case, of chlorine gas entering the titration vessel was therefore avoided. Carbon dioxide was used in the method for economic reasons; oxygen-free nitrogen could also be used.

MR. MOSER commented on the question of impurities, such as iron or titanium, the effect of which had not been considered. The authors had been mainly concerned with pure tin compounds whose stannous and stannic tin content was required for formulation (for new compounds) or for estimation of extent of oxidation (for stannous compounds). They had been able to make all their compounds from high-purity tin metal. It was, in a way, a compliment to the very high quality of tin metal produced by the tin industry that these troublesome impurities had been absent.

DR. W. STROSS asked whether the method was applicable to alloys containing only small amounts of tin. Would titanium, if present in amounts equal to or even greater than the tin content, interfere. What was the lowest concentration of the titrants that could be used.

MR. DONALDSON replied that no attempt had been made to extend the method to the determination of tin in alloys. It should, however, be applicable to alloys that could be dissolved completely in the apparatus in hot concentrated hydrochloric acid. The method would then give tin plus other metals forming reducing cations, *e.g.*, iron and titanium (accurate determination of which was no problem) and hence tin by difference.

Decinormal ceric sulphate had been used for the samples mentioned in the paper; for samples of lower tin content, a more dilute ceric solution could be used. The method was not intended for the determination of trace amounts of tin.

MR. W. J. BAYLEY said that, although the apparatus described provided an ingenious means for carrying out the reduction of tin solutions and the addition of reagent solution without exposure to atmospheric oxidation, it was difficult to see the foundation for the authors' statement that, by existing methods, they could not satisfactorily determine the tin content of pure tin compounds. In general, in the tin-smelting and solder industries, results comparable with those quoted by the authors were obtained by conventional procedures, provided that certain interfering elements were absent; it was only when impurities, such as arsenic, antimony, silver and copper, were present that any real difficulties were encountered.

He said that he would agree with Mr. Okell that the aerial oxidation of stannous chloride during titration with iodine or iodate did not produce errors, provided that the solution was covered with a blanket of carbon dioxide initially and that the titration was not unduly prolonged.

If an accurate method was available for the determination of tin, why refer to it as an empirical standard. Surely metallic tin had all the attributes of purity, stability, etc., required in a primary standard.

MR. MOSER admitted that the conventional procedures gave accurate results in the hands of skilled analysts performing the same titrations frequently and at approximately the same speed. Errors during standardisation against tin metal and during titration were then comparable and largely cancelled out. Such methods could not, however, be applied to occasional determinations, nor could a single empirical standardisation of this kind be applied to samples of widely varying tin content.

Tin metal in these procedures was used as an empirical standard because the method was empirical. The normalities of iodine or iodate solutions thus "standardised" were only applicable to tin determinations by the same procedure and for approximately the same titre. This might be perfectly acceptable in a particular routine analysis. As an academic, he found it much more satisfactory to obtain and use normalities that were still meaningful when applied to other determinations.

The present method had been shown to eliminate all errors due to atmospheric oxidation. By this method, tin could now indeed be used as a primary standard, and would give exactly the same normality for a ceric solution as standardisation against arsenious oxide.

o-Dithiols in Analysis

Part VIII.* Use of the Zinc Complex of Toluene-3:4-dithiol in the Field Testing of Ores and Minerals†

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The zinc complex of toluene-3:4-dithiol is recommended as a reagent for testing for copper, silver, gold, zinc, tin, lead, vanadium, arsenic, antimony, bismuth, molybdenum, tungsten, sulphur (free), selenium, tellurium, manganese, iron, cobalt and platinum in their ores and minerals. By carrying out simple procedures, which are suited to field conditions, most of these elements can usually be detected in the coarsely powdered mineral in 1 minute. The procedures are standardised, as far as possible, to permit the maximum information to be gained from the tests. Interferences are rare and can usually be readily avoided.

It was shown by one of us in previous papers in this series[1,2,3,4] that the zinc complex of toluene-3:4-dithiol (zinc dithiol) can be used in place of toluene-3:4-dithiol (dithiol) as a selective reagent for many thiophilic cations. The tests can be applied rapidly and easily, and the advantages of using a single reagent in place of many are so great that we decided to investigate the use of zinc dithiol in the field testing of ores. Our aim has been to devise simple and rapid preliminary tests for the metals present, which may be of service in the identification of ores.

Almost all the proposed tests can be carried out on the crushed ore in 30 to 60 seconds, except those for noble metals, which take 2 to 3 minutes. The necessary apparatus consists of test-tubes (7·5 cm × 0·95 cm and 12·5 cm × 1·6 cm), a microscope slide, a dropper (teat pipette), a spatula, a polythene wash-bottle and a source of heat. A few common reagents are required.

In most of the tests, zinc dithiol is added to the test solution by means of a spatula. In acid solution, the presence of excess of reagent is revealed at once by the smell of free dithiol. For the few tests in which zinc interferes, a solution of zinc-free dithiol is used, which is prepared as follows—

> To 1 ml of 2 *N* hydrochloric acid in a test-tube add a suitable amount of zinc dithiol. Heat to decompose the zinc complex, add 1 ml of ethylene dichloride, and shake. Insert a plug of cotton-wool, and, by pressing the end of a dropper into its fibres, filter, and remove drops of the lower layer as required.

Filtration, which was resorted to sparingly, was carried out by forcing a piece of cotton-wool down a 7·5-cm × 0·95-cm test-tube and removing the clear liquid with a teat pipette. If the solid was required for testing, the cotton-wool was dried as much as possible by suction, wash liquid was added and the procedure was repeated twice. The colour reaction was then given by the precipitate enmeshed in the cotton-wool.

In the preparation of test solutions, no attempt was made to dissolve ores completely. The proposed tests must therefore be regarded as of a preliminary nature. If, in particular, trace amounts of elements are sought, complete dissolution or attack of the ore would normally be necessary. Nevertheless, in no instance did the extremely rapid treatment cause misleading results.

The procedures used with most ores were as follows—

1. *Preparation of ore*—Reduce a fragment of the ore to powder in a small 1-cm × 2-cm (internal dimensions) percussion mortar. The aim should be to prepare a coarse powder (30 to 80 mesh), which will immediately settle from solution without the need for filtration in subsequent tests.

* For details of previous parts of this series, see reference list, p. 23.
† Presented at the meeting of the Society on Wednesday, November 5th, 1958.
‡ Present address: Department of Geology, University of Leeds.

2. *Preparation of test solution*—Place 1 to 5 mg of the prepared ore in a 7·5-cm × 0·95-cm test-tube. Add 5 drops of concentrated hydrochloric acid, and warm. If there is no immediate sign of reaction, add a trace of potassium chlorate, warm until gas is freely evolved, shake for 10 to 15 seconds and then boil gently for 5 to 10 seconds to remove the smell of chlorine. (Complete removal of chlorine is not necessary.) Allow the undissolved ore to settle, and remove drops of supernatant liquid for testing as required.

The arrangement of the ores tested follows that of Read.[5]

Copper ores

The presence of copper is indicated by dissolving the ore in acid (procedure 2), diluting the solution and adding zinc dithiol; a black precipitate is formed if copper is present.

Tests—

A and B—Mix a few milligrams of ore (prepared by procedure 1) with about twice its bulk of zinc dithiol on a microscope slide. Divide the mixture into two heaps. Moisten one heap with 1 drop of pyridine (test A) and the other with 1 drop of 2 *N* sodium hydroxide (test B). Note any reactions in the cold, and then warm gently. A deep green colour in test A or an orange colour in test B indicates the presence of copper. When there is any doubt, the reaction in test B can be intensified by evaporating the drop to dryness, re-moistening with alkali and re-warming.

Note—A pale yellow colour with no orange tinge in test B indicates sulphur or pyrites (see "Sulphur Ores," p. 21).

Results—

Test A was given in the cold by copper (native), cuprite, tenorite, covelline, bornite, tennantite (slow—1 minute), enargite (slow—1 minute), malachite (extremely rapid) and azurite (extremely rapid). When warmed gently, chalcopyrite, chalcocite, chrysocolla (slow) and atacamite gave the reaction. (Tennanite and enargite also gave the reaction when warmed gently.)

Test B was given by all the ores that gave test A, tetrahedrite, bournonite (somewhat slow), polybasite, stannite (slow), torbernite and an unidentified cobalt ore containing copper, cobalt and a trace of silver (from a disused mine 4 miles N.W. of Leedstown, Cornwall).

In test B, good reactions were given by bournonite, wolframite (containing copper) and stannite when the mixture was dried, re-moistened and warmed a second time.

Silver ores

Tests—

A—Carry out procedures 1 and 2. To 3 drops of test solution add excess of zinc dithiol, and warm. Add water dropwise (2 to 5 drops are necessary), when, in presence of silver, a bright yellow precipitate appears suddenly.

Note—Confusion with antimony, which forms a yellow precipitate at greater dilution, can be avoided (*a*) by heating the liquid, when the antimony complex, but not that of the silver, melts to an orange-yellow oil, or (*b*) by adding a few drops of ethylene dichloride, in which the silver complex is insoluble, but that of the antimony dissolves to give a yellow solution.

Noble metals and tellurium form dark colours with zinc dithiol and interfere in test A. When there is any difficulty, silver is first separated as silver chloride; this is test B.

B—To 3 drops of test solution add 2 to 3 drops of water, and cool. Filter the solution in the way described on p. 16, and wash the cotton-wool twice with 2 *N* hydrochloric acid. Add 2 *N* hydrochloric acid and zinc dithiol, and warm. The cotton-wool is stained yellow in presence of silver.

Nitric acid is often a better solvent for silver ores than is hydrochloric acid; this is the basis of test C.

C—Carry out procedure 1. To a few milligrams of the ore add 3 drops of concentrated nitric acid, and maintain the solution at its boiling-point for 15 to 30 seconds. Add 6 drops of water and zinc dithiol in excess. Re-heat. In presence of silver, the suspended white zinc dithiol suddenly becomes yellow; the yellow precipitate remains unchanged when the solution is boiled.

RESULTS—

Test A was given by cerargyrite. Test B was satisfactory for the complex ores polybasite and sylvanite and when only traces of silver were present, as in the unidentified cobalt ore referred to under "Copper Ores." Test C was more satisfactory than test A for silver (native), stephanite, pyrargyrite and proustite.

GOLD MINERALS

TEST—

Carry out procedure 1. Place 5 to 20 mg of the ore in an ignition tube, and mix with approximately 100 mg each of barium peroxide and barium nitrate. Fuse gently for 30 to 60 seconds, and, while it is still hot, drop the tube into a 15-cm × 1·6-cm test-tube containing 1 ml of 2 *N* hydrochloric acid so that the ignition tube breaks. Boil for a few seconds, pour the liquid into a second tube, add zinc dithiol, and heat. A brownish pink precipitate indicates gold. To confirm, add 0·5 ml of ethylene dichloride, and shake. By means of a dropper, transfer the lower orange layer to a small hard-glass tube. Evaporate the solvent, and heat the residue, which becomes an intense purple. When heated to redness, the glass becomes gilded in patches.

RESULTS—

The test was given by gold (native), sylvanite, calaverite (purple colour not always obtained), petzite and nagyagite.

Sufficient native gold, in the form of grains, could be dissolved in hydrochloric acid and potassium chlorate in 1 minute to give the reaction, but the proposed fusion method was superior.

ZINC ORES

TEST—

Carry out procedures 1 and 2. To 1 drop of test solution add 3 drops of water and sodium acetate crystals in excess. To the cold solution add 5 drops of zinc-free dithiol solution. Hold the tube nearly horizontal, rotate and then shake. In presence of zinc, a bulky white filamentous precipitate forms at the interface, which may cause the ethylene dichloride to appear almost solid.

RESULTS—

The test was given by smithsonite (with darkening), zinc blende and hemimorphite. Lead, manganese and iron commonly occur in zinc ores. The lead complex, being soluble, does not interfere. Small amounts of iron and manganese do not interfere, but iron darkens the precipitate. Cadmium gives the same reaction, but its ores are rare. Most interferences can be prevented by making the solution alkaline, filtering it and then acidifying with acetic acid, but this was not found to be possible within the time limit imposed.

MERCURY ORES

No improvements on existing tests were found.

TIN ORES

TESTS—

A—Carry out procedure 1. Heat a few milligrams of the ore with 1 pellet (0·1 g) of potassium hydroxide and 1 drop of water for 30 seconds. Cool slightly, and add 3 to 5 drops of water. Acidify with 2 *N* hydrochloric acid, add zinc dithiol, and warm. In presence of tin, a red colour or precipitate forms immediately.

B—Carry out procedures 1 and 2. To 3 drops of test solution add 5 drops of water, 3 to 5 drops of ethylene dichloride and zinc dust to neutralise the acid. Add 3 drops of concentrated hydrochloric acid, and warm. Shake well. Add several drops of water and then zinc dithiol. A red colour or precipitate indicates tin.

RESULTS—

Test A was given by cassiterite (samples from Cornwall, Nigeria, Uganda and Malaya). It failed when antimony, indicated by formation of a yellow precipitate that melted when heated, was present. Test B was given by stannite (much antimony and little tin), but, with cassiterite, the colour was pale pink.

Lead ores

Tests—

A—Carry out procedures 1 and 2. To 3 drops of test solution add 5 drops of water and zinc dithiol in excess. If a coloured precipitate forms, maintain at the boiling-point and shake for 10 seconds, during which the precipitate will usually coagulate and leave an almost colourless but often cloudy supernatant liquid. (The cloudiness is caused by the presence of arsenic or an oxidising agent.) Add sodium acetate crystals in excess. In presence of lead, a bright yellow precipitate forms immediately.

Note—The colour of the initial precipitate often indicates the presence of other elements. If the aqueous solution does not rapidly become colourless (as with wulfenite), add an equal volume of ethylene dichloride, warm, shake, and add sodium acetate as before.

B—Carry out procedure 1. Heat a few milligrams of the ore with 1 pellet (0·1 g) of potassium hydroxide and 1 drop of water for 20 to 30 seconds. Add 5 drops of water and then zinc dithiol. A bright yellow precipitate, which forms immediately, indicates lead.

Note—Alkali dissolves sufficient copper from reactive copper ores, *e.g.*, azurite, to give an orange solution with excess of zinc dithiol. If lead is present, however, the lead complex coagulates when heated and collects on the surface of the liquid.

If, after applying the test, the liquid is acidified with hydrochloric acid, the presence of other metals may be indicated, *e.g.*, with wulfenite, the lead precipitate immediately becomes green, thus indicating the presence of molybdenum.

Similar reactions are given by thallium, which is, however, rare in ores.

Results—

Test A was given by galena, minium, cerussite, anglesite, pyromorphite, mimetite, vanadinite, crocoisite, wulfenite, jamesonite, bournonite, nagyagite and a mixture representative of freieslebenite. The slow formation of the tungsten complex made it difficult to achieve a satisfactory rapid reaction with stolzite.

Test B serves immediately to distinguish between non-sulphidic and sulphidic lead ores. It was given by all the non-sulphidic ores that gave test A, including stolzite, but not by the sulphide ores, *viz.*, galena, jamesonite, bournonite and nagyagite.

Vanadium ores

Tests—

A—Carry out procedures 1 and 2. To 3 drops of test solution add 7 to 10 drops of water. Add excess of sodium acetate crystals and a few drops of *iso*amyl acetate. Add zinc-free dithiol solution dropwise, warm gently, and shake. In presence of vanadium, the upper layer darkens and becomes blue, purple or blue-black and almost opaque.

Copper and molybdenum (black and green precipitates, respectively) interfere with this test, but can be removed by dithiol in acid solution, possibly with some loss of vanadium.

B—To 3 drops of test solution add 7 to 10 drops of water and zinc dithiol in excess. Heat, and maintain at the boiling-point for 10 to 20 seconds. Decant or filter the solution, and proceed as in test A.

Results—

Test A was given by carnotite and vanadinite. With vanadinite, a yellow precipitate was also formed, owing to the presence of lead, but it caused no interference. Test B was given by vanadate in presence of nickel and molybdenum (representative of patronite) and of copper and lead (representative of motramite) as well as by solutions containing mixtures of copper, lead and molybdenum.

Cobalt interferes, but is apparently not found in vanadate ores. If suspected, it is easily removed. When a mixture containing copper, cobalt, vanadium^{IV} and iron^{III} in 2 *N* hydrochloric acid was treated with sodium acetate and zinc dithiol, both in excess, the resulting liquid was highly coloured. However, when boiled and shaken, the soluble complexes were decomposed by air and heat in less than 1 minute, and, when the solution was filtered, the filtrate was colourless and gave test A for vanadium^{V}. The removal of iron was incomplete, but the amount remaining did not interfere.

Arsenic ores

Test—

Carry out procedures 1 and 2 (see Note). To 2 drops of test solution add 3 drops of water and 20 to 50 mg of analytical-reagent grade stannous chloride crystals. Heat for 5 to 15 seconds or until the colour of iron^{III} or copper^{II} has been discharged. Immediately add zinc dithiol, and boil for 15 to 20 seconds with shaking. The liquid becomes milky white in presence of arsenic. Pale yellow droplets of the arsenic complex may form on the surface if a large amount of arsenic is present, and the liquid may then redden after a further 10 to 20 seconds, with subsequent precipitation of metallic arsenic.

Note—In procedure 2, insert cotton-wool, and filter if necessary. The presence of undissolved sulphide ore may cause precipitation of stannous sulphide.

Results—

The test was given by arsenic (native), arsenolite, arsenopyrite, cobaltite, enargite, erythrite, mimetite, niccolite, orpiment, polybasite, proustite, realgar, smaltite and tennantite. It was not given by sperrylite (platinum arsenide), which was scarcely attacked in the time allowed. (For detection of arsenic in sperrylite see "Platinum Minerals," p. 22.) Results were negative for all non-arsenical ores tested, including complex ores such as bournonite, jamesonite, sylvanite, pyrargyrite and tetrahedrite.

Antimony ores

Tests—

A—Carry out procedures 1 and 2. To 1 drop of test solution add 3 drops of water. If a precipitate (silver chloride) forms, remove it by filtration. Add zinc dithiol in excess, and heat. An orange-yellow precipitate, which melts to droplets or to a yellow film on the surface and solidifies when cooled to about 80° C, indicates antimony or selenium (see Note).

Note—If little or no reaction occurs during procedure 2, repeat with nitric acid. The concentration of antimony must be fairly high, as the colour intensity given by antimony is weaker than that given by most other elements here considered by a factor of 10 or more.

If desired, selenium (which is rare in ores) may be distinguished by the fact that the reaction with this element is given if zinc dithiol is added *before* dilution of the test solution.

B—Proceed as in test A. If the addition of zinc dithiol gives a dark precipitate (which indicates the probable presence of copper), dilute a further 2 drops of test solution with 2 drops of water, add 2 pellets (0·2 g) of potassium hydroxide, heat to the boiling-point, and filter. Acidify the filtrate with excess of 2 *N* hydrochloric acid, add zinc dithiol, and heat. As in test A, the formation of the yellow complex indicates antimony.

Note—If ethylene dichloride is added to remove the antimony complex from the aqueous layer and sodium acetate is then added in excess, the formation of the brilliant yellow lead complex in the aqueous layer indicates the presence of lead as well as antimony (jamesonite).

Results—

Test A was given by antimony (native), valentinite, antimonite and jamesonite. Test B gave good reactions with bournonite and tetrahedrite, but, with pyrargyrite and polybasite, reactions were weak unless nitric acid was used as solvent (see Note to test A).

Bismuth ores

Test—

Carry out procedures 1 and 2. To 1 drop of test solution add excess of zinc dithiol. Warm, and add 5 drops of water. In presence of bismuth, a dark red precipitate forms. Boil for a few seconds until the liquid is clear. Chill in water. A red colloidal precipitate of the bismuth complex appears, the colour being discharged by heat.

Results—

The test was given by bismuth (native), bismuth ochre, bismutite, bismuthinite and eulytite.

Molybdenum ores

Test—

Carry out procedures 1 and 2. To 1 drop of test solution add 3 drops of water and then zinc dithiol. A brilliant green colour or precipitate forms immediately in the cold.

Note—Tungstate gives no reaction in the cold.

Results—

The test was given by molybdenite and wulfenite. Sufficient molybdenum to give the reaction could be dissolved from a single flake of molybdenite treated with hydrochloric acid (without potassium chlorate) for 30 seconds, although the flake appeared to be unchanged.

Tungsten ores

Test—

Carry out procedures 1 and 2. To 3 drops of test solution add 10 drops of concentrated hydrochloric acid and excess of zinc dithiol. Heat, and maintain at the boiling-point. A blue-green colour develops within 10 to 20 seconds and becomes more intense if the solution is set aside. If molybdenum is present, add 30 to 50 mg of analytical-reagent grade stannous chloride before adding zinc dithiol.[6]

Results—

The test was given by tungsten (metal), wolframite, scheelite and stolzite, none of which contained molybdenum.

Sulphur ores

Test—

Proceed as for copper (test B, p. 17). A pure yellow colour is formed if sulphur is present.

Results—

The test was given by sulphur and iron pyrites, but not by other sulphidic ores that were tested. Copper interferes.

Selenium

When selenium dioxide is sublimed in the vicinity of zinc dithiol, the latter immediately becomes bright yellow. No selenium ores were available, but the element and available compounds (dioxide, potassium selenate and potassium selenocyanate) immediately respond to the following test. Tellurium gives no reaction.

Test—

Mix a trace of the powder or ore with excess of potassium nitrate in a small test-tube. Dust the upper part of the interior of the test-tube with zinc dithiol. Heat to sublime selenium dioxide; the zinc dithiol becomes bright yellow.

For possible confusion of selenium with antimony, see "Antimony Ores," p. 20.

Tellurium ores

Test—

Carry out procedures 1 and 2. To 3 drops of test solution add 3 drops of concentrated hydrochloric acid, 2 to 3 drops of ethylene dichloride and zinc dithiol in excess. Heat, and shake. The drops of solvent darken, become black and take on a characteristic metallic appearance.

Results—

The test was given by tellurium (metal), sylvanite, calaverite, petzite, nagyagite and by mixtures representative of tellurite, tetradymite and hessite.

Manganese ores

Test—

Carry out procedures 1 and 2. To 3 drops of zinc-free dithiol in ethylene dichloride add 3 drops of pyridine and then 1 drop of test solution. An intense dark greenish brown colour (reddish brown in presence of traces of iron) indicates manganese.

Note—If an intense red colour is obtained, iron is present in excess and must be removed. To 3 drops of test solution add excess of ammonium chloride, make alkaline with ammonia, dilute, boil, filter, and add drops of the filtrate to the dithiol in pyridine as described above.

Results—

The test was given directly by pyrolusite and psilomelane, and by a sample of rhodonite after removal of iron.

Iron ores

Iron can be so readily detected by other means that detection by dithiol is in itself of little interest. The following test may, however, be of use in distinguishing between ores, despite the widespread occurrence of iron in ores of other metals.

Test—

Carry out procedure 1. To a few milligrams of ore add 2 drops of zinc-free dithiol in ethylene dichloride and 1 drop of pyridine. A red colour may develop almost immediately in the cold, or only when warmed, or not at all.

Results—

The sulphidic ores pyrrhotite and pyrites formed a red colour instantly in the cold. The carbonate and phosphate ores, chalybite (including ironstone) and vivianite rapidly formed a pink colour on warming. The oxidised and less soluble oxide ores, magnetite (including specular iron), limonite (the sample contained only a trace of iron[II]), hematite and garnet failed to react. For comparison, ferrous sulphate reacted instantly in the cold, and ammonium ferric sulphate slowly developed a pale pink colour on heating.

For the detection of sulphur and cobalt in iron pyrites, see "Sulphur Ores," p. 21 and "Cobalt Ores," respectively.

Cobalt ores

Test—

Carry out procedure 1. Place a few milligrams of the ore and twice its bulk of zinc dithiol in a test-tube. Add 1 drop of pyridine. Warm if necessary. If there is no strong reaction, add 1 drop of 2 *N* sodium hydroxide, and heat to the boiling-point. An intense blue colour indicates cobalt.

Results—

The test was given immediately in the cold by cobaltite (from Cobalt, Ontario). Smaltite and an unidentified cobalt ore (see p. 17) gave a pale blue when warmed and an intense blue after the addition of sodium hydroxide.

Iron pyrites containing cobalt (from Kirembe, Uganda) gave a pale blue colour, which was not intensified by addition of alkali. When a small pile of zinc dithiol was placed on a lump of this ore and moistened with pyridine, the ore became covered with red and blue patches.

Platinum minerals

Test—

Proceed as for gold (p. 18). To a few drops of test solution in dilute hydrochloric acid add 2 to 5 mg of hydroxylamine hydrochloride and excess of zinc dithiol. Maintain at the boiling-point for 1 minute. A violet precipitate, which forms slowly, indicates platinum. Add a few drops of ethylene dichloride, shake, and remove the upper layer. The colour of the solvent (violet) remains unchanged on addition of concentrated hydrochloric acid.

RESULTS—

The test was given by platinum (native—two samples) and by sperrylite. With sperrylite, a marked cloudiness was immediately observed when zinc dithiol was added, which indicates the presence of arsenic. No method of dissolving enough osmiridium in 1 minute to give a satisfactory dithiol reaction could be found.

We thank Mr. P. S. Jewell and Mr. S. F. Everiss of the Cambridgeshire Technical College for their encouragement and help, Dr. S. O. Agrell, Curator of the Museum of Mineralogy and Petrology, University of Cambridge, for his interest and for the supply of many minerals, and Hopkin and Williams Ltd. for a gift of zinc dithiol.

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NOTE—References 1, 2, 3 and 4 are to Parts III, IV, V and VII, respectively, of this series.

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DISCUSSION

DR. J. H. HAMENCE remarked that Dr. Clark had referred to the fact that dithiol was superior to hydrogen sulphide from the point of view of diagnostic tests. In his own experience, this was in fact a very fair statement and was the origin of his paper on the use of dithiol for the detection of molybdenum and tungsten. In the course of some toxicological work, an odd brownish sulphide precipitate had been obtained, which it was thought might be tin, but which when examined by the dithiol reagent gave, not the expected red precipitate, but a brilliant green. The precipitate was subsequently identified as being due to molybdenum.

Clearly, Dr. Clark had produced a useful advance by the introduction of zinc dithiol as the reagent for testing purposes. One of the objections that was frequently raised to the use of dithiol was in respect of its instability and lack of keeping properties. The use of zinc dithiol would obviously remove this snag.

Finally, he asked whether Dr. Clark had worked out any systematic scheme for the detection of metals with dithiol similar to those that had been suggested from time to time with hydrogen sulphide.

DR. CLARK replied that he had made several attempts to do so, but that these had so far been unsuccessful. One of the difficulties arose in connection with iron and cobalt. These metals were easily precipitated by dithiol in weak acid, but with excess of dithiol the precipitates re-dissolved in part, thereby preventing complete separation. Because of the greater cost of dithiol, he thought that at present its more useful role was as a confirmatory reagent. The addition of diacetyldithiol to acid mixtures, in any case, removed most of the difficulties encountered in the precipitation of sulphides.

DR. W. STROSS said that, if he had understood Dr. Clark correctly, the specificity of the reactions consisted in the production of certain colours with certain metals only at certain acid concentrations. As these concentrations seemed to come close to each other (one range being 10 to 6 *N*, the other 6 to 1 *N*), was there not danger of overlaps, and, in consequence, of practical errors.

DR. CLARK replied that although, in the chart he had shown, many cations had for convenience been grouped together within the ranges 10 to 6 *N* and 6 to 1 *N*, no suggestion was intended that cations precipitated at, say, 4 *N* would come down at, say, 2 *N*. This was not so. When dithiol was added to a mixture of two such cations in acid solution, it was, in general, easy to effect a clear-cut separation as shown by the respective colours of the precipitates.

As the colours of precipitates were spread rather evenly over the spectrum and as the complexes were formed under widely different conditions of acidity, any overlap was hard to find. No serious ambiguities arose with the metals discussed in the paper. In the few instances that arose, notably silver and antimony, the properties of the precipitates (solubility in solvent or low melting-point) served to indicate their nature.

DR. R. F. MILTON enquired whether any of the metal dithiols were solvent-soluble and if so would it be possible to use chromatographic methods of separation and determination.

DR. CLARK replied that a number of the complexes were soluble and that doubtless chromatographic methods could be applied. He thought, however, that quicker and better methods of determination, based on the direct addition of dithiol, might be developed and he had already experimented in this direction. However, it was probably desirable to investigate more fully the nature of the complexes formed and the conditions of their formation. A single cation did not always give the same product. Tin gave a number of complexes; at least one was yellow and at least two were red. Thus, in acid solution, according to the conditions, the red product might contain either 2 or 3 atoms of tin combined with 5 dithiol residues. Unless a single type of molecule was formed, chromatography might give misleading results.

Micro-determination of Calcium and Magnesium in Blood Serum and Cerebrospinal Fluid*

By G. HUNTER

(Cowley Road Hospital, Oxford)

Serum and cerebrospinal fluid, deproteinised after suitable dilution by addition of acetic acid and heating, yield filtrates suitable for direct addition to solutions of murexide or Eriochrome black T for the titration of calcium or magnesium. Absolute amounts of about 5 μg of calcium and 0·4 μg of magnesium are present in the test solutions, so that duplicate titrations of both metals can be carried out on 0·2 ml of serum or 0·3 ml of cerebrospinal fluid. As the minimum increment of the standard titrants is 1 drop, the titration for calcium has an inherent error of ± 2 per cent. and that for magnesium $\pm 2{\cdot}5$ per cent.

The "trace metals" of serum may interfere to a slight extent in the determination of both calcium and magnesium. In determining calcium, any potential interference from this source is probably not greater than 1 per cent. and is therefore negligible. In determining magnesium, however, a positive error of about 2·5 per cent. caused by the presence of copper and zinc in the filtrates is probable.

Evidence is given for the speed, accuracy and dependability of the methods, which, on account of their extreme simplicity, are suitable for routine use in hospital laboratories.

The metal indicators murexide and Eriochrome black T have been used by various workers in recent years for the determination of calcium and magnesium in blood, but, despite their apparent theoretical and practical advantages over the older methods, it would appear that they have not been found satisfactory for general clinical use.[1] The methods described in this paper have been used for several thousands of determinations of calcium and magnesium in blood serum and cerebrospinal fluid with consistently good results, and, as they are at once simple and accurate, they appear to be well suited for routine use.

Method

Apparatus and reagents—

The apparatus and reagents are as previously described.[2]

All washed glassware should be passed through a bath of dilute hydrochloric acid, rinsed with distilled water and finally rinsed with de-ionised water. All reagents should be prepared with de-ionised water.

Deproteinisation—

Serum—Place 0·20 ml of serum in a 16-mm × 125-mm test-tube, and add 1·6 ml of water and 0·20 ml of 0·05 *N* acetic acid.

Cerebrospinal fluid—Place 0·30 ml of cerebrospinal fluid in a 16-mm × 125-mm test-tube, and add 2·49 ml of water and 0·21 ml of 0·05 *N* acetic acid. Immerse the tube to just above its liquid level in a bath of boiling water for about 4 minutes. Remove the tube, dry, shake, and spin in a centrifuge for about 5 minutes. Transfer the clear supernatant solution to a clean test-tube with a fine-tipped pipette attached to a "pro-pipette." This test solution is free from protein, just slightly acid and suitable for the determination of calcium and magnesium as described.

In practice, it is convenient to heat together six or more test-tubes held in a suitable rack. Care must be taken to avoid contamination with water from the water bath. For further details, see Hunter.[3]

Procedure for determining calcium—

Carry out the determination as previously described.[2]

In brief, an equal volume of alkaline murexide solution is placed in each cup of the colour comparator and the pointer of the ammeter is adjusted to zero. Then, 0·5 ml of protein-free

* Presented at the meeting of the Society on Wednesday, November 5th, 1958.

serum filtrate, or 1·0 ml of cerebrospinal fluid filtrate, is placed in the right-hand cup and the standard calcium solution is titrated into the left-hand cup until the pointer of the ammeter returns to zero. The amount of calcium present in the test portion is given directly by the burette reading.

PROCEDURE FOR DETERMINING MAGNESIUM PLUS CALCIUM—

Carry out the determination as previously described.[2]

In brief, an equal volume of a buffered solution of Eriochrome black T is placed in each cup, as in the procedure for determining calcium. After adjustment of the pointer to zero, 0·2 ml of serum or cerebrospinal fluid filtrate is placed in the right-hand cup and the standard magnesium solution is titrated into the left-hand cup until the pointer returns to zero. The volume added from the burette is a measure of magnesium and calcium present in the test portion. The amount of calcium present is known from the titration with murexide and the magnesium present can then be read from a calibration graph.

TABLE I

RECOVERY IN PROTEIN-FREE FILTRATES OF CALCIUM AND MAGNESIUM ADDED TO SERA

Serum	Calcium added, mg per 100 ml	Total calcium found, mg per 100 ml	Error, %	Magnesium added, mg per 100 ml	Total magnesium found, mg per 100 ml	Error, %
A	0	8·7	—	0	1·95	—
	2·0	10·6	−1	—	—	—
	4·0	12·8	+1	0·80	2·80	+2
B	0	8·7	—	0	2·00	—
	2·0	10·8	+1	0·40	2·40	0
	4·0	12·4	−2	0·80	2·85	+2
C*	5·0	5·0	0	1·00	0·95	−5
	10·0	10·0	0	2·00	2·00	0
	15·0	14·9	−1	3·00	3·05	+2
D*	5·0	5·2	+4	1·00	1·05	+5
	10·0	9·8	−2	2·00	2·00	0
	15·0	14·5	−3	3·00	3·00	0

* Filtered through Zeo-Karb 215.

RESULTS

It has already been shown[4] by comparison with the results on ashed sera that the method gives accurate results for total serum calcium and magnesium. It can be seen from Table I that the two cations added to sera are quantitatively recovered in the protein-free serum filtrates. Two 10-ml portions of sera were passed through a 1-cm × 15-cm column of Zeo-Karb 215 buffered at pH 7·5. Usual test portions of such sera, deproteinised in the usual manner, show a barely detectable response with the murexide indicator; it is estimated to be less than 1 per cent. of the normal serum calcium. With Eriochrome black T, however, such calcium and magnesium-free sera give definite blank values, commonly equivalent to 0·05 to 0·15 mg of magnesium per 100 ml; that is 2·5 to 7·5 per cent. of that present in normal human sera. Such blank values have been taken into account for sera C and D in Table I. It would appear from Table II that the blank values are probably caused by the presence of copper and zinc. Indeed, the values shown in Table II are not far from those found in the resin-filtered sera.

Most organic substances have some complexing action and ideally should be removed from solutions used for the titration of metals. With deproteinised serum or cerebrospinal fluid, the concentration of organic substances is so low in the test solutions that there is no perceptible difference in the results from filtrates or from solutions of ashed sera. There is, however, sufficient protein in the test portion of diluted whole serum necessary for both calcium and magnesium titrations to cause extremely low results for both metals. This is shown very simply in Table III. It is therefore surprising that several workers have described methods, especially for the determination of calcium in serum with murexide as indicator, without removal of protein.

Our clinical results with the present methods will be described in other reports, but it seems of some interest to record here (see Table IV) our magnesium results alongside those obtained by Denis's method[5] on the same sera in the Biochemistry Department, Central

TABLE II

PROBABLE EFFECTS OF THE "TRACE METALS" OF SERUM ON THE DETERMINATION OF CALCIUM AND MAGNESIUM

The assumption is made that the serum contains 10 mg of calcium and 2 mg of magnesium per 100 ml and that, respectively, 0·05 and 0·02-ml test portions are used

		Determination of calcium with murexide as indicator			Determination of magnesium with Eriochrome black T as indicator		
			Probable interference—			Probable interference—	
Metal	Assumed level of metal in serum, μg per ml	Observed calcium equivalent to 1 μg of metal, μg	from 0·05 ml of serum, μg	on basis of 5 μg of calcium, %	Observed magnesium equivalent to 1 μg of metal, μg	from 0·02 ml of serum, μg	on basis of 0·4 μg of magnesium, %
Copper ..	1·0	0·6	0·03	0·6	0·6	0·012	3·0
Iron	2·0	0	0	0	0·1	0	0
Manganese ..	0·03	0	0	0	0·8	0	0
Zinc	1·6	0	0	0	0·3	0·006	1·5

TABLE III

COMPLEXING EFFECT OF PROTEIN IN THE DETERMINATION OF CALCIUM AND MAGNESIUM IN SERUM

Sera and filtrates diluted (1 + 9)

	Determination of calcium with murexide as indicator—		Determination of magnesium with Eriochrome black T as indicator—	
Serum No.	in serum, mg per 100 ml	in protein-free filtrate of serum, mg per 100 ml	in serum, mg per 100 ml	in protein-free filtrate of serum, mg per 100 ml
1	8·2	9·8	1·50	1·95
2	8·6	9·6	1·80	2·10
3	8·2	9·6	1·65	2·10
4	8·2	8·8	1·65	2·15

TABLE IV

SERUM MAGNESIUM FOUND BY DIFFERENT METHODS IN BOVINE HYPOMAGNESAEMIA

Serum No.	1	2	3	4	5	6	7	8
Magnesium found by Denis's method at Weybridge, mg per 100 ml	0·7	0·5	0·4	0·7	0·3	1·1	1·1	0·6
Magnesium found by proposed method at Oxford, mg per 100 ml	0·5	0·4	0·5	0·8	0·2	1·1	1·2	0·6

TABLE V

MAGNESIUM IN PLASMA FROM HEPARINISED BLOODS AGEING AT ABOUT 4° C

	Magnesium found in plasma after—					Increase in approximately 7 days, %
Subject	0 hours, mg per 100 ml	21 hours, mg per 100 ml	68 hours, mg per 100 ml	118 hours, mg per 100 ml	166 hours, mg per 100 ml	
B	1·72	1·95	2·05	2·22	2·50	45
C–R	1·34	1·52	1·75	1·85	1·90	42
L	2·04	2·20	2·50	2·55	2·70	32
J	1·92	2·02	2·30	2·35	2·45	28
M	2·06	2·10	2·35	2·67	—	—

Veterinary Laboratory, Weybridge, in the obscure condition of bovine hypomagnesaemia. With one exception, there is good agreement between the two quite distinct methods, even at the very low magnesium levels.

The bloods used for calcium and magnesium determinations should be freshly taken, and the serum or plasma separated from the cells as soon as possible. Human erythrocytes

contain about 2·5 times more magnesium than the corresponding serum[6] and lose magnesium to plasma, as already noted by Cohen,[7] when whole blood is stored. The increase of serum magnesium is shown in Table V by the five heparinised bloods left to age in a refrigerator. Even within 24 hours an increase could be observed in all of them.

A slight haemolysis in sera makes little or no difference to the results, as the haemoglobin iron is removed with the protein, but markedly haemolysed blood will obviously give erroneously high results.

Discussion of the method

The colour comparator described permits the determination of the end-point, with murexide as indicator, to an accuracy of $\pm 0{\cdot}1$ μg of calcium and with Eriochrome black T as indicator to an accuracy of $\pm 0{\cdot}01$ μg of magnesium.

In the use of this instrument, it should be noted that the colour of the solutions in the opposite cups should be visually similar at both the beginning and end of the titrations (it is possible to set the ammeter to zero with solutions that do not match visually). Small differences in volume of the buffered indicators are compensated for by the initial zero adjustment without introducing an error in the subsequent titration, but, if the ammeter is set to zero with solutions of different spectral qualities, then not only will the solutions not match at the end-point, but the titration will be inaccurate. Initial contamination of the indicator solutions can arise from unexpected sources, *e.g.*, traces of saliva in pipettes.

The ubiquity of calcium and magnesium calls for some care in the collection of fluids, but in the course of analysis of several thousand samples we have seldom met with extraneous metals.

Some of the advantages of the present method may be noted. The method of deproteinisation of serum or cerebrospinal fluid yields faintly acid filtrates suitable to add, without neutralisation, to the metal indicator solutions. The very low concentration of acetic acid present has no appreciable complexing action, and the need for blank tests is avoided. No metals are added to the filtrates during deproteinisation.

The high dilutions at which calcium and magnesium are titrated precludes, by a large margin, any complexing action by phosphate, citrate or other serum or cerebrospinal fluid constituent.

The titration of magnesium without removal of calcium with oxalate has proved a great convenience and gives more precise results than are possible with the use of ethylenediaminetetra-acetic acid for reasons already stated.[2] The methods have proved to be simple and reliable and need no unusual care or skill to carry out. Duplicate analyses for both calcium and magnesium can be completed on 0·2 ml of serum in about 30 minutes, although it is usually more convenient to work with batches, as has been stated.

As the minimum increment in the titration for both calcium and magnesium is 1 drop of standard solution, equivalent to $0{\cdot}1$ μg of calcium and $0{\cdot}01$ μg of magnesium, with the test portions used there is an inherent error of ± 2 per cent. for calcium and $\pm 2{\cdot}5$ per cent. for magnesium.

I thank the Biochemistry Department, Central Veterinary Laboratory, Weybridge, for supplying hypomagnesaemic sera. I also thank Mrs. K. M. Nunn for technical assistance.

The work described is part of a programme in the development of methods for the study of the blood - cerebrospinal fluid barrier in association with Dr. H. V. Smith, Reader in Medicine in the University of Oxford, and is made possible by a grant from the Nuffield Foundation.

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Discussion

Dr. R. F. Milton congratulated the author on producing an extremely elegant piece of analytical work. He felt that, for the first time, we would be able to obtain accurate levels of blood calcium and magnesium. The old Kramer and Tisdall method for blood calcium had stood since 1921 and heaven knows what the results meant. Equally for magnesium, precipitation as triple phosphate and subsequent colour estimation of the phosphate was filled with pitfalls, and to be able to obtain results to the second place of decimals by Hunter's method really was an achievement.

Mr. D. D. Moir asked whether, in the instrument used for determining the end-point, the two tubes had to be filled accurately to the same mark or volume, or whether this was immaterial.

Dr. Hunter replied that the volumes in the two tubes need not be equal, as the light beam passed through the length of the liquid column, as in the visual comparison of coloured fluids in Nessler tubes. It was this fact indeed that made the titration procedure feasible.

Determination of Demeton-methyl Residues in Plant Material

By E. Q. Laws and D. J. Webley

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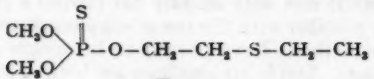
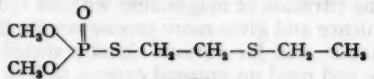
A method is given for isolating demeton-methyl from naturally occurring phosphorus compounds in vegetables. The quantitative evaluation of the insecticide is described and the treatment of some growing crops is discussed. Some results obtained by measurement of cholinesterase inhibition are compared with the analytical figures.

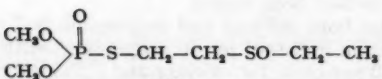
Demeton-methyl, known also as metasystox, occurs in two isomeric forms in the commercial product. These are the thionate (**I**) and the thiolate (**II**). Compound **II** by oxidation in the plant gives a sulphoxide (**III**) and a sulphone (**IV**). Compound **II** is about four times



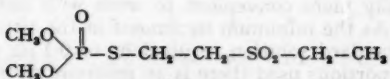
(**I**)



(**II**)



(**III**)



(**IV**)

as toxic as compound **I** and the toxicity of **III** and **IV** is of the same order as that of **II**. There is also a sulphonium compound[1] resulting from the conversion of **II** that has up to 100 times the toxicity of **III**.

The transformation of **I** to **II** is rapid in aqueous solution. In the plant, conversion to **III** is very rapid and oxidation to **IV** is slow. Compound **IV** is finally decomposed to orthophosphoric acid. Compounds **I**, **II**, **III** and **IV** are extracted from water by chloroform while the sulphonium compound remains in the water.

All our results are based on chloroform extraction from an aqueous or partly aqueous solution and will in consequence take account of substances **I**, **II**, **III** and **IV**, but will not include the highly toxic sulphonium compound. Whether this is of major significance in determining the toxicity of demeton-methyl residues in plants is at present unknown. It appears from the results of Heath and Vandekar[1] that, although the sulphonium compounds have a high toxicity when injected into animals and show this toxicity in anticholinesterase experiments *in vitro*, they are no more toxic than the parent thiolate when taken orally. It is likely that the results obtained chemically or by anticholinesterase activity on the chloroform extracts represent to a fair approximation the toxic hazard to the consumer of the treated crop.

When the chemical analysis of traces of systemic insecticides in plant material by phosphorus determination is under consideration, the crux of the problem is the separation of the insecticide from naturally occurring plant substances that contain phosphorus. The difficulty of this separation varies from one plant to another and also depends on the nature of the insecticide. Methods that have been used to effect this separation are solvent extraction,[2] distillation[3] and chromatography.[4] Studies have been made of the distribution of systemic insecticides in plants by means of labelled phosphorus. This is not applicable to the determination of residues in plant materials that have been treated with commercial non-radioactive insecticide. In consequence, the determination of phosphorus after removal of the insecticide from interfering phosphorus compounds remains the most satisfactory chemical approach to the problem of insecticide residues. Alternatively, the measurement of anticholinesterase activity may well be of major importance. With this in mind, some experiments on the measurement of cholinesterase inhibition were undertaken by Mr. E. I. Johnson and Mr. R. D. A. Polhill of this laboratory, who used the method of Hensel *et al.*[5] as modified by Boyd.[6]

When the measurements are made on aqueous extracts of the plants, some extracts, notably those of potato haulm and lettuce leaves, give high blank values. The blank value for potato is of a much higher order than that for lettuce. When the figures obtained by anticholinesterase measurements on chloroform extracts are compared with those by the chemical method, there is reasonable agreement and the blank values are low.

The necessity for finding methods of analysis for new insecticides as quickly as possible results in an empirical approach to each one, whereas what is really required is a systematic study of the chromatography of vegetable extracts so that the separation of a new insecticide could be predicted from the known chromatographic behaviour of solutions of the pure material. No attempt is made in this paper to reach this ideal, but the experience gained in the study of demeton-methyl residues may be of use in a general approach to other insecticides.

Experimental

Extraction—

The extraction of the insecticide is carried out by macerating the shredded plant material in aqueous methanol and then washing the extract with light petroleum to remove some of the phosphorus compounds. The insecticide is then extracted from the aqueous portion with chloroform. At this stage, the chloroform still contains some unwanted phosphorus compounds. Potatoes, lettuces and cabbages give satisfactory results, but Brussels sprouts are troublesome on account of phosphorus compounds that are not completely removed by the subsequent chromatography and, in consequence, give a high blank value. To overcome this difficulty, the initial maceration of the sprouts is carried out in *iso*butyl alcohol. The extract is then shaken with water and light petroleum, whereupon the insecticide goes into the water, the interfering phosphorus compounds being removed by the light petroleum. Blank values obtained by this procedure are equal to the reagent blank, but the insecticide recoveries are not as complete as in the first method. In the light of these results, it was decided to keep the *iso*butyl alcohol method for "difficult" crops and to adopt the methanol - water system as the standard procedure. The chloroform solution from either extraction is concentrated to a small volume and forms the solution for chromatography. The demeton-methyl at this stage is mostly in the form of the sulphoxide or the sulphone, the proportion depending on the time between treatment and examination.

Chromatography—

With the low molecular weight compound tetramethylphosphorodiamidic fluoride (dimefox), chromatographic separation of the insecticide from interfering natural substances is easy, a column of magnesia[4] being sufficient to hold back all the natural phosphorus compounds while the insecticide is eluted with ether. With the more complex molecules of demeton-methyl and its derivatives, the separation is more difficult and in all instances when columns of polar adsorbents, such as magnesia, calcium carbonate or alumina, are used, the insecticide is either held on the column or, if a solvent of sufficient polarity to elute the insecticide is used, interfering phosphorus compounds are present in the eluate.

These difficulties are overcome by using a column of a non-polar adsorbent (active carbon). With such a column, elution of all the insecticide with but little impurity is found to be possible for most vegetables. If with a particular vegetable, as, for example, sprouts,

the solution prepared by the water - methanol extraction procedure fails to give a satisfactory blank value after chromatography, extraction by the *iso*butyl alcohol method is used followed by chromatography on the carbon column. Under these conditions, the blank value is reduced to a minimum and the recovery of demeton-methyl is reasonably good. We have studied only cabbage, lettuce, potatoes and sprouts. These observations may not hold for all plants.

Method

The method consists in the extraction of the demeton-methyl derivatives from the plant material, in which they are present mainly as the sulphoxide and the sulphone, their separation both by selected extraction methods and chromatography from interfering compounds and the subsequent determination of the phosphorus by spectrophotometric measurement of the molybdenum blue complex.

Apparatus and reagents—

The apparatus and reagents are as described by Field and Laws.[4] In addition, the following reagents are required—

Chloroform B.P. (*phosphate free*).

iso*Butyl alcohol*—Laboratory-reagent grade (phosphate free).

Active carbon—Mesh 14 to 22, otherwise as described by Hancock and Laws.[7]

Standard solutions—

These were prepared from samples of the sulphoxide (**III**) and the sulphone (**IV**) of approximately 90 per cent. purity obtained from Baywood Chemicals Ltd.

Prepare a standard solution by dissolving approximately 0·1 g of the compound in 1 litre of water, and dilute the solution as required to give approximately 10 μg of the compound per ml. Standardise by reference to potassium dihydrogen orthophosphate.

Sampling—

Prepare a representative sample of the plant material and shred it to as fine a condition as possible by means of a domestic slicing and grating machine. Sub-sample 50 g of the shredded material, taking account of the distribution of any liquid, and extract by procedure A or B.

Extraction—

Procedure A—Macerate the 50-g sub-sample with 100 ml of methanol - water (1 + 1) for 15 minutes, and filter the mixture by suction through a Whatman No. 1 filter-paper on a Buchner funnel. Wash the residue with a further 50 ml of methanol - water, and transfer the combined filtrates to a 500-ml separating funnel. Rinse the filter-flask with 100 ml of water and then 100 ml of light petroleum. Shake the combined filtrate and washings, and separate the layers, breaking up any emulsions by means of a centrifuge if necessary. Discard the upper light petroleum layer and wash the methanol - water layer with three further 100-ml portions of light petroleum. For potato tubers, one additional washing is sufficient. Extract the aqueous layer four times with 60-ml portions of chloroform. Discard the aqueous solution and evaporate the combined chloroform extracts to a small volume in a conical flask.

Procedure B (*for use when procedure A gives a high blank value*)—Macerate the 50-g sub-sample with 100 ml of *iso*butyl alcohol. Filter the mixture as described for procedure A, and wash with a further 50 ml of *iso*butyl alcohol. Add 150 ml of light petroleum to the combined *iso*butyl alcohol filtrates in a separating funnel and extract five times with 50-ml portions of water. Reject the green organic layer and combine the water extracts. Wash the combined water extracts twice with 75-ml portions of light petroleum, and reject the petroleum layers. Extract the aqueous solution four times with 60-ml portions of chloroform, combine the chloroform extracts, and evaporate in a conical flask to a small volume.

Chromatography—

The carbon column—Use a tube fitted with a glass tap, as described by Hancock and Laws.[7] Insert a cotton-wool plug into the chromatographic tube and add sufficient chloroform to cover the plug. Pour in a slurry of 3·5 g of the active carbon in chloroform and insert another plug of cotton-wool on the top of the column. Transfer the chloroform solution for analysis to the column and rinse the flask three or four times, adding the rinsings to the

column. Elute with a total of 100 ml of chloroform at a rate of approximately 2 ml per minute into a 250-ml conical flask or, if an accurately measured aliquot is required, as for example, when a sample of the eluate is taken for anticholinesterase determination, into a stoppered graduated cylinder.

The eluate—Evaporate the eluate, or an aliquot of it, in a 250-ml conical flask and immediately dissolve the residue in 10 ml of water. If extraction procedure B was used, add 2 ml of concentrated nitric acid and 0·5 ml of concentrated hydrochloric acid, and boil to dryness before adding the nitric, hydrochloric and perchloric acids as in the method of Field and Laws.[4] This is a safety precaution to avoid bringing *iso*butyl alcohol and fuming perchloric acid together. Proceed from this point to the final phosphorus determination.

Results

Standard materials—

The experimental results are based on samples of the commercially pure sulphoxide and sulphone. Unless otherwise stated, they are expressed in terms of the sulphoxide, as this is the form most likely in plants that have been recently treated with demeton-methyl. The same procedure is adopted for the anticholinesterase figures.

Both the sulphoxide and the sulphone were of 88 to 90 per cent. purity based on figures for chloroform-soluble phosphorus.

Limits of detection—

The amount of vegetable matter taken for each individual determination is usually 50 g. The blank value for the complete extraction procedure on this amount, including all reagents, is an average optical density of 0·030, which corresponds to 3·8 μg of sulphoxide. If we take a figure of twice the blank value as the limit of detection, this gives a value of 7·7 μg or 0·15 p.p.m. It is clear that smaller amounts than this can be detected, but the reliability of the result decreases as the blank value is approached, owing to variations in the blank values from individual experiments.

Recoveries—

Recovery of the sulphoxide and the sulphone in the chloroform extraction is between 90 and 100 per cent. Recovery from plant material that has been subjected to the complete procedure is of the order of 80 to 90 per cent. of the chloroform-soluble portion. This is shown by the results in Table I, which shows recovery values for the two compounds added separately to spring cabbage that had been grown without the use of insecticides. Similar figures were found for other crops, notably potato tubers, cauliflower leaves and Brussels sprouts.

Table I

Recovery of added insecticide from cabbage

Amount of insecticide added, μg	Amount of insecticide found, μg	Recovery, %
Addition of sulphoxide to 25-g portions of shredded cabbage—		
Nil	Nil	—
11·7	10·6	90
17·6	15·6	90
23·4	17·7	76
29·2	22·8	78
Addition of sulphone to 25-g portions of shredded cabbage—		
Nil	Nil	—
6·7	6·1	93
13·5	12·6	93
20·2	16·3	80
26·9	23·8	88
33·7	28·6	85

Field trials—

The crops chosen for field trials were spring cabbage, lettuce and potatoes.

Samples of the untreated plants were taken just before spraying with the commercial insecticide. Further samples were taken at intervals and subjected to analysis. The interval between sampling and extraction was kept as short as possible and was usually 1 day, or,

at the most, 2 days. The experiments were carried out in duplicate, and, for lettuces and potato haulm, anticholinesterase values of the chloroform extracts were measured on the same solutions as were used for the phosphorus determinations. The results of these field trials are shown in Table II.

Table II

Field tests on cabbage, lettuce and potato haulm

Each test was carried out on a 50-g sample. Potato haulm gave troublesome emulsions and high blank values

Amount of demeton-methyl applied per acre, fluid ounces per 100 gallons of spray	Time of sampling after spraying, days	Amount of insecticide found, as sulphoxide, p.p.m.	Mean amount of insecticide found, p.p.m.	Sulphoxide anticholinesterase value, p.p.m.
Spring cabbage sprayed on April 14th, 1958—				
	0	11·2, 7·4	9·3	—
	1	6·9, 2·3	4·6	—
	3	3·7, 4·1	3·9	—
12	10	3·3, 2·1	2·7	—
	21	0·36, 0·38	0·37	—
	30	0·10, 0·09	0·10	—
	Control	Nil, Nil	<0·04	—
Lettuce sprayed on July 16th, 1958—				
	0	2·6, 2·1	2·4	3·7
	1	4·1, 3·4	3·8	3·6
	3	1·7, 1·7	1·7	1·8
6	10	1·3, 1·0	1·1	1·3
	21	0·2, 0·2	0·2	Nil
	30*	—	—	—
	Control	—	<0·06	Nil
Potato haulm sprayed on June 16th, 1958—				
	0	6·5, 4·6	5·6	9
	1	4·3, 2·0	3·2	4·3
	3	2·2, 2·7	2·5	1·9
24	10	0·15, 0·25	0·20	1·0
	21	0·03, 0·03	0·03	Nil
	Control	—	0·15	Nil
	7 (Tubers)	Nil	Nil	—

* No sample available; crop ploughed in.

Discussion of results

The figures in Tables I and II refer to known additions of insecticide to vegetables and also to unknown amounts added by standard commercial spraying practice to growing crops. In all instances, satisfactory blank values are obtained and the insecticide is evaluated with reasonable accuracy. The fair agreement of the chemical figures and those obtained by anticholinesterase measurement is additional confirmation of the accuracy of the method.

We would like to thank all who have helped us in this work, in particular, the Directors of Rothamsted Experimental Station, the Scottish Horticultural Research Institute and the Plant Pathology Laboratory, Harpenden, for their ready co-operation in supplying us with treated crops and control samples, and the Government Chemist for permission to publish this paper.

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The Determination of Iodine-131 in Urine

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Aliquots from 24-hour specimens are oxidised by heating under reflux with acid potassium permanganate, which also converts iodine present to iodate. The iodate is reduced by oxalic acid to iodine, which is distilled, extracted into carbon tetrachloride and then into water containing sulphur dioxide. Finally, the iodine is precipitated as silver iodide for counting.

A BIOLOGICAL monitoring programme involving analysis of urine was envisaged to cover those handling iodine-131. Personnel dealing with iodine-131 work in laboratories with efficient extracts and glove boxes. Since, under these conditions, the body absorption of only small amounts of radioactive iodine could be expected, the use of a large portion of a 24-hour sample was likely to be necessary.

The maximum permissible level of iodine-131 in the body is 0·6 μc.[1] If the excretion rate is assumed to be 0·1 per cent. per day, the maximum permissible level in urine would be 600 $\mu\mu$c, *i.e.*, 1332 disintegrations per minute per 24-hour sample. With a counter efficiency of 8 per cent., this represents 107 counts per minute per 24-hour sample. It was required to detect iodine-131 to below 10 $\mu\mu$c per 24-hour sample (*i.e.*, less than 1·8 counts per minute above background with a method efficiency of 100 per cent.).

EXISTING METHODS—

A gamma spectrometer has been used to determine iodine-131 in urine; the method involves pouring urine into a polythene cup that fits over the sodium iodide crystal of the counter. The amount of urine that can be used is dependent on the crystal size. Although this method is rapid, instruments to bring the level of accurate determination to below 50 $\mu\mu$c are expensive, and the method, although attractive, is beyond the scope of this paper. A relatively cheap but efficient method for iodine determination was required and existing chemical methods were examined.

In the Weybridge method mentioned by Bratt for the determination of iodine-131 in biological material, the sample is oxidised by an acidic potassium permanganate solution and then reduced by oxalic acid. With the apparatus specified,[2] gases from the distillation flask pass through a sodium hydroxide trap into a reflux condenser. The condensate is then returned via a small-bore tube to the distillation flask. As water condenses on passing through the trap, a "head" is built up, which subsequently becomes greater than that in the reflux-return connection, and the vapour ceases to bubble through. The trap contents can be run off and replaced by more sodium hydroxide, but this is obviously unsuitable when large volumes of urine are to be dealt with.

The Walton and Johns method for iodine-131 in grass has been modified for application to urine by Bratt; the method consists in chromic acid oxidation and then reduction with oxalic acid. A trap flask and four gas-washing bottles containing carbon tetrachloride are connected to a distillation flask. Although the collection of iodine in this way is satisfactory, it was found to be rather cumbersome.

Harington's nickel-dish method[3] (a modification of Kendall's method[4]) consists in heating the urine in a large excess of sodium hydroxide, the iodine being retained as sodium iodide. The method is convenient for small volumes of urine, but not for volumes of about 1 litre. Evaporation of the urine to a suitable volume would obviously result in an uncertain loss of iodine.

A method[5] has recently been described by Fletcher for the separation of organic from inorganic iodine in urine. A silver chloride column is used to absorb inorganic iodine and an ion-exchange resin to concentrate organic iodine in the remaining liquid; a chromatographic separation follows. In normal urine analysis for iodine-131 this is an unnecessary refinement, as only the total iodine is required.

The existing methods proved to be of little value, except for that of Walton and Johns, since relatively large volumes of urine were involved. It was therefore decided to modify part of Walton and Johns's method to develop a more practical procedure for iodine collection.

Experimental

A 24-hour sample is collected initially. This permits accurate assessment of the level of radioactivity in a 900-ml aliquot and overcomes, to some extent, variations in excretion of the isotope from the body. Twenty milligrams of potassium iodide in 10 ml of water are added before oxidation to act as carrier.

Although the majority of the iodine in urine is in the inorganic state, up to 20 per cent. may be organic, as thyroxine or di-iodotyrosine.[5] It was therefore necessary in determining total radioactive iodine to include a preliminary oxidation to bring it all into the inorganic state. An acid permanganate oxidation was used, as this has the advantage of distinct colour change when the permanganate has been totally reduced. Efficient refluxing was necessary, owing to the possible volatility of iodine, and, for this purpose, a double-walled condenser was used.

Twenty milligrams of potassium iodide carrier give a theoretical yield of 28·3 mg of silver iodide, and recovery was checked by weighing the precipitate after it had been dried. The yield varied between 21 and 24 mg of silver iodide, which corresponds to chemical yields of from 75 to 85 per cent.; the results of four experiments were as follows—

Weight of silver iodide, mg ..	21·2	24·0	23·2	22·8
Yield, %	75	84·7	81·8	80·4

After oxidation, the iodate formed was reduced to iodine by oxalic acid, and the iodine and water were removed by distillation in the apparatus shown in Fig. 1. A small amount of carbon tetrachloride was put into the 1-litre bottle to prevent iodine adhering to the joints. The distillation was continued until a temperature of 120° C was attained; a large volume of water and the iodine were collected in the 1-litre bottle. Any iodine that passed this bottle was retained by sodium hydroxide solution in a Dreschel bottle through which the remaining gases were bubbled.

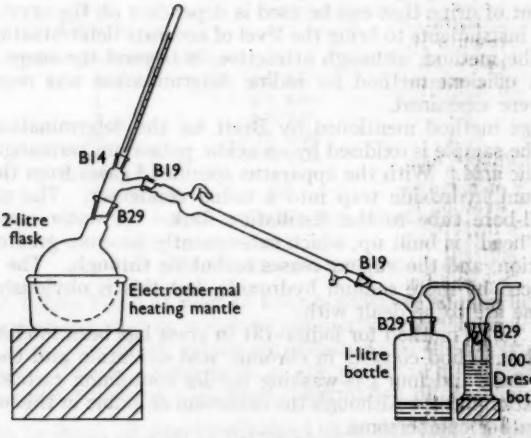

Fig. 1. Distillation apparatus

In this method, all the distillate is collected, whereas, in the modified Walton and Johns method, water is distilled into a trap and run off before the iodine distils. It was often found that some iodine distilled with the water; the latter method could therefore lead to a loss of iodine.

Towards the end of the distillation, a small amount of chlorine is evolved. It is slightly soluble in carbon tetrachloride and the small amount absorbed passes through the subsequent stages and is present in the final precipitate. This is not serious, as any radioactive chlorine present in the urine is diluted with at least 20 g of inactive chlorine per litre. The only chlorine isotopes that are beta-emitters have half-lives of less than 1 hour except chlorine-36 (4×10^5 years). Only large amounts of active chlorine isotopes present in the urine would therefore make any appreciable difference to the radioactivity of the final precipitate.

Bromine-82, if present in urine, is the only other beta-emitting halide likely to interfere, although, again, only large amounts would have any effect, as its half-life is 35 hours and the precipitate is not usually counted until 4 days after voiding.

It was found that a 1-litre Dreschel bottle could adequately replace a gas-washing train as used in the Walton and Johns method, the over-all method efficiency still being 80 per cent. The bottles are easy to set up and to wash. A trap was incorporated in the Dreschel bottle to prevent sodium hydroxide from being sucked back during distillation.

The contents of the first bottle were transferred to a separating funnel and acidified with nitric acid. Sodium nitrite was added to oxidise any iodide present to iodine, which was extracted with carbon tetrachloride. The contents of the second bottle were acidified and similarly treated. The combined extracts were shaken with water containing sulphur dioxide, which absorbed the iodine. This extract was made alkaline with sodium hydroxide solution, the mixture was boiled to remove sulphur dioxide, acidified with dilute nitric acid and the iodide was precipitated as silver iodide by adding dilute silver nitrate solution. The precipitate was coagulated, washed with dilute nitric acid, water and acetone and mounted on an aluminium counting-tray.

In Bratt's modification of the Weybridge method, the precipitate was mounted by using a polythene tray-holder. This consisted of two pieces of polythene with circular holes slightly less than the diameter of the perforated aluminium tray cut in them. These were clamped together, with the tray in between, by four screw-clips in such a way that the tray was gripped firmly and evenly around the whole of its rim. This assembly was attached to a rubber stopper with a hole in it, and the stopper was placed in the neck of a Buchner flask attached to a vacuum pump, with the tray in a horizontal position. The precipitate could thus be collected on a filter-paper placed in the perforated tray by applying suction.

It was found that the precipitate adhered to the polythene walls round the rim of the tray. Also, when washed, the precipitate tended to be absorbed into the filter-paper, which resulted in an unpredictable fall in radioactivity, as beta-particles were absorbed by the filter-paper.

The method of mounting finally adopted was to use a teat-pipette, the tip of which had a bore of 2 to 3 mm. With a little practice, 95 per cent. transference to the tray could be achieved; this was checked by weighing the precipitate on the tray. This method gave superior recoveries, although the precipitate did not have as flat a surface as that produced by the polythene-holder method.

Method

Add 20 mg of potassium iodide in 10 ml of water (as carrier) to 900 ml of a 24-hour sample of urine in a 2-litre flask. Add 125 ml of concentrated sulphuric acid and then 25 g of potassium permanganate, slowly and with cooling. (Add the permanganate a little at a time, as the initial oxidation is usually vigorous.) Set the flask aside for 1 hour to allow the initial reaction to subside, and swirl the contents occasionally during this period. Add a few glass beads, and place the flask in an electric heating-mantle. Heat under reflux, gently at first (to minimise frothing), for 4 hours. Use a double-walled reflux condenser, surmounted by an expansion adapter, the lower half of which contains loosely packed glass-wool supporting a few grams of activated charcoal. (The gas trap was found to be necessary when carrying out the determination in the open laboratory, as chlorine is evolved during heating. In view of the oxidising conditions, free iodine is unlikely to be evolved, but, if any should be evolved, it will be condensed in the efficient reflux condenser.) If the solution becomes colourless during the heating period, cool, and add more potassium permanganate.

Allow the solution to cool to 60° C, and add oxalic acid until the colour just disappears. (Ignore any brown precipitate in the flask; this usually dissolves during distillation.) Set up the apparatus for distillation, as shown in Fig. 1. Place 30 ml of carbon tetrachloride in the 1-litre bottle and 50 ml of 7 *N* sodium hydroxide in the Dreschel bottle. Distil until the temperature of the evolved vapour reaches 120° C. (Most of the iodine and a considerable amount of water collect in the 1-litre bottle.) Cool, and transfer the contents of the 1-litre bottle to a 1-litre separating funnel. (After passage through the condenser, the distillate may still be quite warm; if it is not cooled, excessive pressure builds up in the separating funnel when carbon tetrachloride is added.) Rinse the bottle and add the rinsings to the separating funnel. Add 2 ml of 5 *N* nitric acid and 1 g of sodium nitrite in 10 ml of water.

Shake, allow to separate, and run off the carbon tetrachloride layer containing the iodine. Extract with 10-ml portions of carbon tetrachloride until the solvent layer is colourless.

Transfer the sodium hydroxide solution from the Dreschel bottle to a 1-litre separating funnel, dilute with 300 ml of water, and add 40 ml of carbon tetrachloride. Acidify the mixture with *N* nitric acid (about 350 ml), add 1 g of sodium nitrite in 10 ml of water, shake, and run off the solvent layer. Repeat the extraction with 10-ml portions of carbon tetrachloride until the solvent layer is colourless. Combine the carbon tetrachloride extracts with those of the distillate from the 1-litre bottle.

Wash the combined extracts with 30 ml of water, and discard the water. Dilute a few drops of water that have been saturated with sulphur dioxide to 10 ml, and shake the solvent with this solution. Repeat this extraction until the solvent layer is colourless; combine the aqueous extracts. Filter the aqueous solution into a 50-ml centrifuge tube, and make alkaline with 7 *N* sodium hydroxide. Place the tube in a bath of hot water for a few minutes to expel sulphur dioxide. Cool the solution, acidify with *N* nitric acid, and add 1 per cent. silver nitrate solution until precipitation is complete.

Place the tube in a bath of hot water to coagulate the precipitate. Spin in a centrifuge, wash the precipitate once with 0·1 *N* nitric acid, three times with water and once with acetone. Spin in a centrifuge after each washing. (The presence of a small amount of wetting agent, *e.g.*, sodium dioctyl sulphosuccinate, is useful during these operations.) Form a slurry of the precipitate with acetone, and mount on an aluminium tray by using a pipette fitted with a rubber teat and having a tip of bore 2 to 3 mm. Count the sample with a conventional beta-counter that has been calibrated by using a standard silver iodide precipitate (prepared from iodine-131) and the same weight of carrier.

Efficiency of the method

The efficiency of the method was determined by adding equal volumes of an iodine-131 solution to the sample and to a solution of 20 mg of potassium iodide. After acidification with nitric acid, silver nitrate solution was added to the latter solution and the precipitated silver iodide was mounted on a counting tray. This was taken as a standard, and the procedure was repeated at various levels of radioactivity. Typical results are shown in Table I.

Table I

Recovery of iodine-131

The time of counting in each experiment was 2 hours

Source of precipitate	Count	Background count	Count corrected for background	Counts per minute	Recovery, %	Mean recovery, %
With a standard of ~ 130 μμc—						
Standard ..	35,316	273	35,043	292·6	—	—
Samples ..	29,112	249	28,863	240·5	82·5	79·9
	28,003	305	27,698	230·8	79·0	
	27,280	357	27,023	228·2	78·1	
With a standard of ~ 20 μμc—						
Standard ..	6013	298	5715	47·6	—	—
Samples ..	4664	351	4313	35·8	76·6	81·3
	4993	362	4631	38·4	82·1	
	5010	210	4800	40·0	85·5	
	4823	341	4482	37·3	79·7	
With standards of ~ 1 μμc—						
Standard ..	550	273	277	2·31	—	—
Samples ..	520	251	269	2·24	97·0	82·7
	540	305	235	1·80	76·6	
	510	287	223	1·86	80·6	
	473	257	216	1·80	76·6	
Standard ..	510	270	240	2·00	—	—
Samples ..	444	273	171	1·45	72·5	75·1
	489	291	198	1·65	82·5	
	432	280	152	1·27	63·5	
	473	273	200	1·67	83·5	

The standard deviation at the 1-$\mu\mu$c level is equivalent to $\pm$10 to 15 per cent. of the activity. This could of course be reduced, if required, by increasing the counting time.

With an over-all method efficiency of 80 per cent. and a counter efficiency of 8 per cent., 10 $\mu\mu$c of iodine-131 were equivalent to 1·4 counts per minute. With a background of 3 counts per minute and a 2-hour count, the method permitted satisfactory detection down to 0·2 counts per minute, which is approximately 1/400 of the maximum permissible level.

I thank Mr. G. N. Walton, Chemistry Division, A.E.R.E., Harwell, for checking the initial draft and for helpful suggestions.

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An Ion-exchange Method for the Separation of Thorium from Rare Earths, and its Application to Monazite Analysis

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Thorium is adsorbed on a strongly basic anion-exchange resin, Amberlite IRA-400 (sulphate form), from a sulphate solution of pH about 2·0. Under these conditions, tervalent cerium and other rare earths are adsorbed to only a slight extent. An ion-exchange method has been developed for the separation of thorium from these elements. The method has also been applied to the determination of thorium and rare earths in monazite concentrates.

Thorium is generally associated with rare-earth elements in its minerals and hence considerable interest has been shown in its separation from these elements. Moeller, Schweitzer and Starr[1] reviewed the various methods used for this purpose, most of which involve selective precipitation of thorium by inorganic or organic reagents. More recently, organic solvents, either alone[2] or in conjunction with cellulose columns,[3] have been used for extracting thorium from a nitrate medium. Although ion-exchange resins have been successfully used in the separation of individual rare earths from one another, their use for the separation of thorium from rare earths has not attracted much attention. Radhakrishna[4] used Amberlite IR-100, a cation-exchange resin, for the separation of thorium from lanthanum. He used radioactive tracers and studied their separation by adsorbing them on a short resin column and then eluting lanthanum and thorium with ammonium citrate and 6 *N* sulphuric acid, respectively. When a separation of 50 mg each of thorium and lanthanum oxides was attempted in this laboratory, it was found that the elution of the two elements was not efficient. Gordon, Firsching and Shaver[5] described an ion-exchange method for concentrating microgram amounts of rare earths present in a relatively large amount of thorium. The method is based on preferential complexation of thorium with ethylenediaminetetra-acetic acid, but does not give a complete separation of rare earths and thorium.

While our work was in progress, Danon[6] reported on the adsorption of thorium from 7 *N* nitric acid on anion-exchange resins, but did not test the behaviour of rare earths under the same conditions.

The proposed ion-exchange method is based on the observation that, from sulphate solution, thorium is adsorbed on a strongly basic anion-exchange resin, *e.g.*, Amberlite IRA-400, whereas rare earths are not.[7] The adsorbed thorium can be easily eluted by dilute acids. The existence of anionic complexes of thorium in sulphate solutions does not seemto have been reported in the literature.

Experimental

Reagents—

Technical-grade reagents were used to prepare solutions of thorium sulphate (4 mg of thorium oxide per ml) and cerous sulphate (10 mg of ceric oxide per ml). Dilute cerous solutions were prepared from this stock solution. A solution for eluting adsorbed cerium was prepared by acidifying distilled water to pH 2 with sulphuric acid.

Resin—

Amberlite IRA-400, consisting of 36 to 50-mesh B.S.S. particles, was completely converted to the sulphate form by passing a 5 per cent. w/v solution of sodium sulphate through a column packed with the resin. The resin was then rinsed with distilled water until the effluent was free from sulphate, removed from the column and stored wet in a bottle.

Ion-exchange columns—

The 15-mm internal diameter ion-exchange columns were of the conventional type. Enough resin to give the necessary depth of bed was added in the form of a slurry and was allowed to settle. The column was then rinsed with 50 ml of acidified water. The rate of flow of the solutions passing through the column was controlled by operating the stopcock of a separating funnel fitted at the top of the column to serve as a reservoir.

All pH adjustments were made with the help of a Cambridge bench-type pH meter.

Adsorption and elution of thorium—

Preliminary experiments indicated that the adsorption of thorium was sensitive to pH changes in the feed solution, the break-through capacity of the column decreasing as the pH decreased. Since at pH values above 3·0 there was a danger of thorium being precipitated as basic sulphate and at pH values below 1·0 the adsorption of thorium was too poor to be of much practical use, the sample solution, before passage through the column, was always adjusted to a pH between 2·0 and 2·5.

The adsorbed thorium could be eluted with 2 *N* nitric, hydrochloric or perchloric acid.

Method for separating thorium from cerium

Cerium was chosen to represent the rare earths of its group because of the ease with which it could be determined, and its separation from thorium was tested by the proposed method. Preliminary experiments showed that tervalent cerium from a sulphate solution was adsorbed to a small extent by the resin under the same conditions as thorium, but the adsorbed cerium could be washed from the column by passing a sufficient amount of acidified water (pH 2).

Procedure—

Dilute the solution containing thorium and tervalent cerium as sulphates to about 75 ml, and adjust the pH to about 2·3 by adding a little sulphuric acid or sodium hydroxide solution. Prepare an ion-exchange column (10-cm bed) as described previously, and pass the solutions through it at a rate of 2·5 ml per minute. Wash the column with 200 ml of acidified water (pH 2) at a rate of 5 ml per minute. Combine the effluent and washings, and determine cerium colorimetrically[8] or volumetrically.[9]

Elute thorium with 150 ml of 2 *N* hydrochloric acid at a rate of 2·5 ml per minute. Use ammonium hydroxide to precipitate thorium from the effluent, filter the mixture, and dissolve the precipitate in the minimum amount of dilute hydrochloric acid. Determine thorium by precipitation as oxalate.[10]

Effect of other ions

As an anion-exchange resin is used, metal ions that do not form anionic complexes cause no interference in the adsorption of thorium. Many of the common cations are in this category. To confirm this, 100 mg each of certain cations, as sulphates, were mixed

separately with aliquots of thorium sulphate solution containing 100 mg of thorium, as ThO_2; the cations were—

$$Be^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+}, Cu^{2+}, Ni^{2+} \text{ and } Co^{2+}.$$

The separation and determination of thorium was carried out in the way described for solutions containing cerium; none of these ions was adsorbed to any detectable extent.

IRON—

Ferric iron tends to be adsorbed on the resin with thorium. When a solution containing 50 mg of Fe^{3+}, as ammonium ferric sulphate, and 100 mg of thorium oxide was passed through a column, about 5 mg of ferric iron were adsorbed; this was eluted with the thorium. However, the final thoria residue contained a negligible amount of iron, as the latter was removed during the oxalate precipitation. The adsorption of iron could be practically overcome by reducing the solution with sulphur dioxide before passing it through the column.

URANIUM—

Uranium is known to form a sulphate complex, which is adsorbed by the anion-exchange resin.[11] Although it is not possible to separate uranium from thorium by the proposed method, the presence of amounts of uranium up to 50 mg, as U_3O_8, can be tolerated, as it is removed during the oxalate precipitation of thorium.

COMMON ANIONS—

Since anions compete with the thorium complex for resin sites, premature break through of thorium is caused if they are present in appreciable amounts. Under the conditions described, however, 50 mg each of nitrate and chloride ions and 1·0 g of sulphate ion, as the sodium salts, caused no interference.

APPLICATION OF THE METHOD TO MONAZITE ANALYSIS

To test the applicability of the method to the separation of thorium from the rare earths present in monazite, samples of monazite concentrates that had been previously analysed for thorium and rare earths were taken.

As a sulphate solution is required for ion-exchange separation, decomposition of monazite by heating with concentrated sulphuric acid, one of the standard methods, would have been ideal. However, as monazite is a phosphate mineral, it was difficult to adjust the resulting sulphate solution to a pH between 2·0 and 2·5, owing to precipitation of thorium and rare earths as phosphates. To overcome this difficulty, the sulphate solution must be phosphate-free, and a procedure was adopted for decomposing monazite and converting thorium and rare earths to a sulphate solution for ion-exchange separation.

PROCEDURE—

Decomposition of monazite[12]—Accurately weigh 0·5 to 1·0 g of powdered monazite into a platinum crucible, and mix it with 5 to 6 g of potassium hydrogen difluoride. Heat the crucible, gently at first, until the fluoride melts, and maintain it at that temperature until the melt is clear. Cool, and transfer the fused mass to a platinum dish with about 100 ml of hot water. Add 2 to 3 ml of hydrofluoric acid, break any remaining lumps with a platinum rod, and place the dish on a steam-bath for 15 to 20 minutes. Allow the precipitate, which consists mainly of the fluorides of thorium and rare earths, to settle, and filter the supernatant liquid through a Whatman No. 40 filter-paper. Wash the precipitate by decantation with 20 ml of water and 0·5 ml of hydrofluoric acid. Repeat the washing five or six times. Ignite the filter-paper and the small amount of precipitate in a platinum crucible at low heat to destroy all carbon. Transfer the residue to the platinum dish containing the bulk of the precipitate, add 5 to 6 ml of concentrated sulphuric acid, and heat until fumes are evolved.

When sufficient acid remains to keep the sulphates in the form of a thick paste, cool, and extract with about 100 ml of ice-cold water containing a few millilitres of 5 per cent. sulphurous acid solution to reduce any oxidised cerium. If the solution is not clear after 1 to 2 hours, filter, and ignite the residue at dull red heat. Add a few drops of sulphuric acid, heat until fumes are evolved, dissolve the sulphates as previously described, and combine the sulphate solutions.

Separation of thorium from rare earths—Adjust the pH of the sulphate solution to 2·0 by careful addition of concentrated sodium hydroxide solution. If the solution is yellow, oxidised cerium is present; this can be reduced by adding a few milligrams of sodium nitrite.

Prepare an ion-exchange column (20-cm bed) with the sulphate form of the resin, as described previously, and pass the sample solution through at a rate of 2·5 ml per minute. Rinse the column with 250 ml of acidified water at a rate of 5 ml per minute. Combine the effluent and washings, and stir in 30 g of powdered oxalic acid to precipitate the rare earths as oxalates. Set the mixture aside overnight, filter, ignite the oxalates, and weigh as mixed rare-earth oxides.

Elute thorium from the column with 200 ml of 2 *N* hydrochloric acid at a rate of 2·5 ml per minute, and determine as described under "Method for Separating Thorium from Cerium."

Discussion of results

Tables I and II show results for the separation of synthetic mixtures of thorium and cerium and for the separation of thorium and rare earths from monazite. From these results, it can be concluded that the method gives satisfactory separation of thorium from cerium and other rare earths of this group. In some of the experiments with thorium and cerium mixtures, the thoria residues were fused with potassium hydrogen sulphate and cerium was then determined colorimetrically.

Table I

Separation of thorium and cerium from synthetic mixtures

Amount of thorium present, as ThO_2, mg	Amount of thorium found, as ThO_2, mg	Amount of cerium present, as CeO_2, mg	Amount of cerium found, as CeO_2, mg	Amount of cerium found in thoria residue, as CeO_2, mg
100·0	100·5	—	—	—
40·0	39·8	—	—	—
100·0	101·0	100·0	98·6	—
40·0	40·2	5·0	5·0	0·05
40·0	39·7	5·0	4·8	0·05
20·0	20·2	200·0	198·0	0·30
20·0	19·5	200·0	201·0	0·22

Table II

Separation of thorium and rare earths from monazite

Amount of thorium present, as ThO_2, %	Amount of mixed rare-earth oxides present, %	Weight of sample, g	Amount of thorium found, as ThO_2, %	Amount of mixed rare-earth oxides found, %
8·60	60·7	0·5	8·60	—
		0·5	8·68	59·8
		0·5	8·69	59·9
		1·0	8·56	60·4
		1·0	8·45	60·5
		1·0	8·60	61·0
10·00	62·0	1·0	10·0	62·2
7·56	58·5	1·0	7·70	59·4

The primary object of the investigation was to test the method for the separation of thorium from rare earths, but the behaviour of a number of ions was also studied and the results showed that satisfactory separation of thorium could be achieved in presence of a number of common cations. The presence of an excess (more than 1·0 g) of free sulphate was found to reduce thorium adsorption. Although in experiments with thorium and cerous sulphate mixtures the amount of sulphate present was nearly equivalent to the metals present, this was not so for the monazite samples, as some free sulphuric acid always remained when the fluorides were converted to sulphates. This was found to lead to premature leakage of thorium in the effluent during adsorption. For this reason, a 20-cm resin bed was used in monazite analysis, although a 10-cm bed was satisfactory for synthetic thorium - cerium mixtures.

We thank Dr. Jagdish Shankar, Principal Research Officer, Atomic Energy Establishment, Trombay, and Dharma Veer Bhatnagar for their encouragement and helpful suggestions during the course of the work.

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Rapid Micro-determination of Organically Bound Halogens, Arsenic, Phosphorus and Boron

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Rapid micro methods for the determination of organically bound chlorine, arsenic, phosphorus and boron have been devised, based on a combustion procedure demonstrated by Schöniger.

After combustion in oxygen, chloride is titrated electrometrically with silver nitrate, arsenic iodimetrically as trichloride, phosphorus alkalimetrically after precipitation as quinoline molybdophosphate and boron as the mannitol complex with sodium hydroxide, the carbon dioxide being swept out by an inert gas.

At the International Congress on Microchemistry in Vienna in 1955, W. Schöniger of Basle demonstrated an elegant method for the determination of organically bound halogens and sulphur,[1] based on previous work by Hempel[2] and Mikl and Pech.[3,4] The method seemed too simple to work, but trial proved that it did, even in inexperienced hands. Moreover, it seemed likely that, with suitable modifications, the method could be used not only to determine halogens and sulphur, but also arsenic, phosphorus and boron. The procedures adopted for these last-named elements are now described, together with some observations on the determination of chlorine.

In the method as demonstrated, the weighed sample was rolled in a small piece of filter-paper and placed in a platinum clip, which was then suspended from the stopper of a 250-ml conical flask previously filled with oxygen. The filter-paper was ignited and plunged back into the flask, where it burned very brightly for a few seconds. According to Schöniger, a temperature of about 1200° C could be attained momentarily. The products of combustion were absorbed in a few millilitres of water in the bottom of the flask, the final determination being made on this solution.

Before the details of the various procedures are described, it should be stated that for each determination only a few standard substances have been examined, and it may be that extremely stable compounds do not break down completely. For example, it was found that individual particles of resins exploded during combustion and some fragments escaped complete decomposition; results were therefore low and variable.

Micro-determination of arsenic

Preliminary work showed that, although the arsenic-containing compound was burned in an excess of oxygen, arsenite as well as arsenate was formed. Since distillation of arsenic as trichloride would sometimes be necessary to remove interfering elements, it was decided as a general practice always to follow this course after reduction to the lower valency state. Some difficulty was encountered in choosing the reducing agent best suited to the conditions. As results with ferrous iron and stannous chloride were unreliable, a statement by Kobrová[5] that hydrazine sulphate was a suitable reducing agent led to the use of hydrazine dihydrochloride, and results were at once satisfactory.

The Pyrex-glass apparatus used was similar to that described by Horton, Thomason and Miller[6] for the distillation of fluorine, except that the thermocouple pocket and steam generator, being unnecessary for arsenic, were omitted (see Fig. 1).

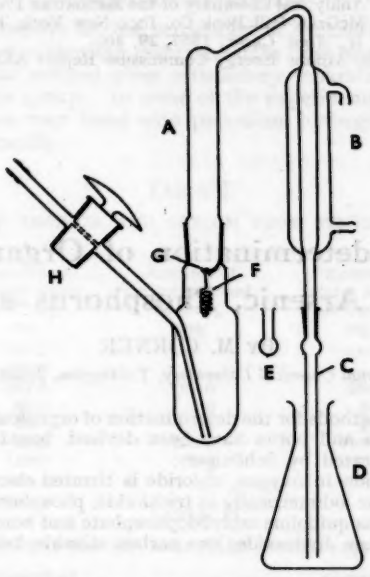

Fig. 1. Apparatus for the determination of arsenic

Procedure—

As the volume of the flask was only about 100 ml, it was necessary to reduce the oxygen consumed by the wrapping paper to a minimum; half a cigarette paper, weighing about 20 mg, proved to be satisfactory. The weighed sample was rolled in the paper, which was then threaded through silica spiral F to leave a small tail as touch-paper, the spiral being hung from glass hook G fused to the lower end of air condenser A. The flask, containing 2 ml of aqueous sodium hydroxide (1 pellet in 2 ml), was flushed out with oxygen through the side-arm. The tap was then closed and the lower end of water condenser B was covered with ground-glass cap E. The touch-paper was ignited and the condenser quickly replaced in the neck of the flask while the sample burned fiercely. A time of 5 to 10 minutes was allowed for complete absorption of the products of combustion. The cap was then replaced by tube C, which dipped in a saturated solution of sodium bicarbonate in flask D, and the combustion chamber was heated so that the spiral and sides were washed down by the condensate. After neutralisation to phenolphthalein with concentrated hydrochloric acid introduced through side-arm H, 3 ml of water containing approximately 0·1 g of hydrazine dihydrochloride and 0·03 g of potassium bromide were similarly added and then 5 ml of concentrated hydrochloric acid. The tap was closed and the mixture was heated to boiling; the arsenic trichloride distilled over into the bicarbonate solution in about 7 ml of distillate, which was then titrated with 0·01 *N* iodine (1 ml ≡ 0·3746 mg of arsenic) to a faint blue colour, starch being

used as the indicator. During this titration, solid sodium bicarbonate was added to remove the hydriodic acid formed. Another 5 ml of concentrated hydrochloric acid were added to the combustion vessel and a further portion was distilled into the blue solution. Any arsenic trichloride not removed by the first distillation will decolorise the bicarbonate solution and more iodine must be added to restore the blue colour. The reaction proceeds in accordance with the following equation—

$$AsCl_3 + 2HCl + I_2 = AsCl_5 + 2HI.$$

When resin samples were analysed, the difficulty caused by decrepitation was overcome by carrying out the determination as described, but without the absorbing liquid in the combustion vessel; any unburnt particles were destroyed by carefully heating the bottom of the flask. After the flask had cooled, the absorbing liquid was introduced through the side-arm and the determination was continued as described. Some results that agreed satisfactorily with the expected figures are shown for the last two compounds in Table I.

Esters of arsenious or arsenic acids gave better results if merely distilled from concentrated hydrochloric acid, the combustion stage being omitted.

TABLE I

DETERMINATION OF ARSENIC

Compound	Weight of compound taken, mg	Arsenic found, %	Arsenic calculated, %
p-Aminophenylarsonic acid	3·452	34·56	34·53
	4·428	34·53	
	5·233	34·47	
	3·848	34·52	
	2·782	34·45	
Research sample NS74	2·778	36·18	
	3·512	35·92	
Research sample AJH2	3·223	10·77	
	3·963	10·76	
Styrene polymer	2·719	6·92	
	3·828	6·91	
Research sample G14 (dry combustion)	5·186	26·66	
	5·723	26·32	

MICRO-DETERMINATION OF HALOGENS

CHLORINE—

Although the determination of halogens and sulphur by Carius's method has always proved satisfactory, it seemed that the adoption of the method demonstrated by Schöniger might result in a saving of time. As it was proposed to extend the method to liquid and volatile samples, the problem of a suitable container had to be solved. The only capsules obtainable commercially were of gelatin; these were satisfactory with regard to size and ease of handling, but could be objected to on the grounds that (*a*) being of animal origin they might not be chemically uniform, (*b*) they soften easily when wetted, and (*c*) they might be soluble in some compounds. In spite of these disadvantages, they were used at first with the standard compound *o*-chlorobenzoic acid and the results are compared with those for the weighed sample enclosed in half a cigarette paper (see Table II).

TABLE II

DETERMINATION OF CHLORINE IN *o*-CHLOROBENZOIC ACID

Theoretical chlorine content of *o*-chlorobenzoic acid = 22·68 per cent.

Chlorine found after lamp ignition in—		Chlorine found after flame ignition in paper wrapping with—	
paper wrapping, %	gelatin capsule, %	no carbon added, %	carbon added, %
23·20, 21·52 (Mean 22·36)	22·95, 23·33, 21·32, 22·38, 22·92, 21·97, 20·57 (Mean 22·21)	19·38, 19·08, 22·26, 19·80, 19·70, 18·36, 19·70 (Mean 19·75)	22·55, 22·90, 22·69, 22·82, 22·34, 22·44, 22·52, 22·11, 22·62, 22·13, 22·19 (Mean 22·44)

The apparatus used was a 250-ml round-bottomed flask having a B14 stopper drawn out to a hook at the lower end, from which a platinum sample-holder was hung. The weighed sample was enclosed in half a cigarette paper or a gelatin capsule, and combustion was started either by lighting a fuse or by energy from a 1000-watt tungsten lamp placed at one of the foci of an ellipsoidal reflector, the sample being placed at the other. This latter method was discarded later as being unsuitable for volatile compounds. The combustion products were absorbed in a few millilitres of water and titrated electrometrically with 0·01 *N* silver nitrate. Results were low, presumably owing to the formation of chlorine or oxides of chlorine. According to Gibbs,[7] chlorine will combine with water in the presence of carbon to produce hydrochloric acid even at room temperature, in accordance with the following equation—

$$2Cl_2 + C + 2H_2O \longrightarrow 4HCl + CO_2.$$

Carbon black (about 20 mg) was therefore added to the aqueous absorption solution, and further results were satisfactory.

Four samples, the chlorine contents of which were unknown to the analyst, gave good results, except for the compound containing 41 per cent. of halogen. The results for this substance were satisfactory when twice the usual amount of carbon black was added (see Table III).

TABLE III

DETERMINATION OF CHLORINE IN UNKNOWN SAMPLES IN PRESENCE OF CARBON

Sample	Chlorine calculated, %	Chlorine found, %	Chlorine found in presence of twice the usual amount of carbon, %
A	14·20	14·34, 14·09	
B	15·58	15·67, 15·61	
C	13·45	12·89, 13·14	
D	41·23	40·23, 38·80	41·45, 41·04
		39·94, 37·94	41·54

For the determination of halogen in liquid and volatile samples, the need for capsules made from a material likely to be more chemically uniform than gelatin became urgent, and the choice fell on adhesive cellulosic tape, which gave a low blank value proportional to the amount of tape used. A strip 1 cm × 2 cm was lined with a smaller piece of chromatographic paper strip laid on the tape so that a sticky border remained on two long sides and one short side. The tape was then rolled lengthwise, with the filter-paper lining on the inside, round a glass rod, starting at the end partly covered by filter-paper. One end was sealed, and the capsule so formed was withdrawn from the rod and weighed. A small amount of the liquid sample was dropped on the filter-paper from a capillary, the second end was sealed and the whole was re-weighed. At first, the capsule was held in an 80-mesh platinum-gauze basket, but the gauze frequently developed holes; it was replaced by a platinum spiral having a turn brought across the bottom to prevent the sample from slipping through.

Ten determinations were made on chloroform. The results varied from 84·7 to 90·5 per cent. of chlorine, the average being 87·4 per cent.; the calculated value is 89·1 per cent. Further work is being carried out along these lines.

MICRO-DETERMINATION OF PHOSPHORUS

With phosphorus compounds as with arsenic, a silica spiral or basket had to be used. The combustion was carried out as already described, and the products of combustion were absorbed in water. Cigarette paper gave a high blank value and even 50 mg of chromatographic paper strip together with the reagents gave a blank value of about 0·5 ml of 0·1 *N* sodium hydroxide (1 ml ≡ 0·1192 mg of phosphorus), which agrees with the blank values obtained by Wilson in the original method. The combustion gave rise to some reducing body that converted the molybdophosphate to the blue compound, and so the absorption solution was evaporated down with 1 drop of nitric acid before the phosphorus was precipitated as quinoline molybdophosphate by Wilson's method. The yellow precipitate was collected on a sintered-glass filter-stick, washed with dilute hydrochloric acid (1 + 9) and water, and then dissolved in standard sodium hydroxide, the excess of alkali being titrated

back to slight acidity with 0·01 *N* hydrochloric acid; carbon dioxide was boiled out and, when cool, the solution was brought to pH 8, a glass electrode being used as indicator. The reaction proceeds in accordance with the following equation—

$$(C_9H_7N)_3H_3[PO_4.12MoO_3] + 26NaOH = Na_2HPO_4 + 12Na_2MoO_4 + 3C_9H_7N + 14H_2O.$$

Triphenyl phosphine was used as the standard compound because it had a somewhat similar structure to the compounds to be analysed. The phosphorus content is 11·81 per cent.; the results of duplicate analyses were 11·75 and 11·6 per cent. Although a number of research samples were examined, the results were variable. Other analytical results also varied, and so the samples were regarded with suspicion.

Since the completion of this work, a paper by Flesher, Southworth, Hodecker and Tuckerman on "The Determination of Phosphorus in Organic Compounds. Rapid Micro- and Semi-micro Method"[9] has been published, in which use is made of the method demonstrated by Schöniger, but the final procedures differ from those described in this paper.

Micro-determination of boron

Wet oxidation with potassium persulphate was first examined, but in our hands gave unacceptable results; once again combustion in an oxygen-filled flask was tried and proved satisfactory.

The samples to be analysed were expected to be of the borazol type and it seemed probable that boron nitride might be formed. In order to prevent this, the weighed sample was mixed with about a similar bulk of finely ground potassium hydroxide and the mixture was wrapped in a strip of cellulosic sheet, 3·5 cm × 2 cm, weighing 20 to 30 mg, as this was found to burn more readily than filter-paper. The combustion was carried out in the usual manner. The decomposition products, together with the alkali residue on the platinum spiral, were shaken with a few millilitres of distilled water until completely dissolved. This solution was washed into a 50-ml squat beaker, the final volume being 20 to 25 ml, and brought to pH 3 with hydrochloric acid; carbon dioxide was blown out by bubbling oxygen or other suitable gas through the solution (see Jackson and Bailey[10]). The gas was allowed to flow for a few minutes and then the pH was raised to 5·5 by the addition of sodium hydroxide. With the oxygen still bubbling through, 3 to 4 g of mannitol were added and the solution was titrated to pH 8·0 with 0·1 or 0·01 *N* sodium hydroxide according to the boron content, this titre being equivalent to the boron present.

1 ml of 0·01 *N* sodium hydroxide ≡ 0·1082 mg of boron.

Table IV shows the percentages of boron found in sodium tetraphenylborate, phenylboronic anhydride and four research samples by this method.

Table IV

Determination of boron

Compound	Amount of compound taken, mg	Boron found, %	Boron expected, %
Sodium tetraphenylborate	21·288	3·18	3·16
	11·281	3·24	
Phenylboronic anhydride	20·708	9·94	10·41
	9·842	9·94	
Research sample $P_2/29/B/L$ ..	10·530	7·77	7·86
	10·769	7·75	
Research sample $P_1/145/D/T$* ..	3·838	14·41	14·35
	5·632	13·88	
Research sample $P_2/153/D/W$ ..	5·803	14·43	14·35
	4·861	14·45	
Research sample $P_2/151/D/T$† ..	3·979	19·03	19·7
	5·071	19·05	

* A borazol type compound.
† Results by an independent worker who had no previous experience with the method.

The work described has been carried out as part of the research programme of the National Chemical Laboratory, and this paper is published by permission of the Director of the Laboratory.

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A Field Method for Determination of Lead Fume

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A field test for determining small amounts of lead fume in industrial atmospheres is based on the formation of a purple colour on test paper impregnated with tetrahydroxy-*p*-benzoquinone. After collection on the test paper, lead particles are dissolved *in situ* by means of a spray of acetic acid and acetone. The lead fume forms a uniform purple background stain, which is compared with a standard colour chart; isolated pin-points of more intense colour may also be formed by larger particles of lead dust. By this means, it is possible to avoid the error, inherent in ordinary chemical methods, of including adventitious lead dust in the figure for lead fume. Means of overcoming the effect of interference by other metal fumes are discussed.

FUMES are usually defined as solid condensed dispersoids of particle diameter less than 1μ. Lead fume is composed mainly of lead oxide particles or, more rarely, when formation has taken place in the absence of air, of lead particles. The mean particle size is well below 1μ.

Several methods are available for the determination of lead in the atmosphere. They involve collection of the lead by drawing a sample of air through a suitable mechanical filter, an impinger or an electrostatic precipitator. The lead is then dissolved or extracted from the filter and analysed in the laboratory by an appropriate method. Such methods, although accurate, are time-consuming, and there is a need both for a field method for determining total air-borne lead and for a field method whereby the lead content of a sample of lead fume collected on a filter medium can be immediately determined by a simple treatment of this medium.

Feigl and Suter[1] described the use of sodium rhodizonate in conjunction with a buffer solution of sodium hydrogen tartrate and tartaric acid for detecting lead by the formation of a coloured complex. Amdur and Silverman[2] used this buffer solution and the more stable tetrahydroxy-*p*-benzoquinone to develop colour stains with air-borne lead particles collected on a filter-paper. We describe a field method in which tetrahydroxy-*p*-benzoquinone is used for the determination of lead fume in the presence of moderate amounts of lead dust. The method of developing a stain is simple and rapid.

EXPERIMENTAL

SOURCE OF LEAD FUME—

A stream of air was directed on to the surface of lead powder contained in a continuously agitated vessel. The finer particles of dust that were carried over were fed into the air inlet of an air - gas burner. The lead fume produced by combustion was collected in a large glass vessel, and, after an interval of a few minutes, mixed with an air stream and passed through a test paper.

Other samples were taken by passing air or nitrogen through an electrically heated furnace containing a vessel of molten lead. In the field, samples were taken at various points in a lead-refining works and in a factory manufacturing storage batteries.

DEVELOPMENT OF STAIN—

Although the collection and determination of particulate matter in the field can be largely effected by the technique used for toxic gases and vapours, additional problems are raised by the heterogeneous nature of fumes and dusts.

A successful field test for lead fume must fulfil the following requirements in the development of the stain—

(*i*) the efficiency of collection on the test paper must be reasonably high,
(*ii*) the particles must be uniformly distributed over the test paper and this uniformity of distribution must not be disturbed by the procedure for developing the stain,
(*iii*) the lead fume must be converted on the paper to a form with which the reagent will combine to give the stain,
(*iv*) the sensitivity and range of the method should be adequate, and the change in intensity at each step of the standard colour chart should be sufficient,
(*v*) no fading should take place during the period required to match the colour of the stain,
(*vi*) the stain must be confined within the boundary of the standard area of test paper, and
(*vii*) the stain should be formed substantially on the surface of the test paper to permit visual assessment of the colour.[3]

It was not possible to realise all these objectives by using an aqueous solution of tartaric acid - sodium hydrogen tartrate applied dropwise, as recommended by Amdur and Silverman,[2] whose object was to determine the total air-borne lead particulate matter and not specifically lead fume. Accordingly, the lead fume was dissolved on the test-paper surface by means of a glacial acetic acid - acetone spray, whereupon a stain was developed within a few seconds.

SELECTION OF FILTER-PAPER—

La Torre and Silverman[4] state that a filter-paper used for sampling air-borne materials in the field should (*a*) show a relatively high collecting efficiency, (*b*) have uniformity in collecting efficiency from paper to paper, and (*c*) have a low uniform resistance to air. Smith and Surprenant[5] have also examined the efficiency of various papers.

Munktell No. 00 filter-papers were found to give satisfactory results in the field test. They have an average collecting efficiency of about 90 per cent. and a resistance to air of 0·6 inches of water per sq. foot per cubic foot of flow rate per minute.

It was found that some brands of filter-paper otherwise suitable, *e.g.*, Whatman No. 2, after treatment with an ethanolic solution of tetrahydroxy-*p*-benzoquinone turned a light yellow colour, which interfered with the subsequent comparison of stains. The formation of the yellow colour could be avoided by previously washing such papers successively with *N* acetic acid and distilled water and drying them in an air oven.

PARTICLE SIZE AND TOXICITY—

Inhalation is considered to be the most serious mode of exposure to lead poisoning in industry. The precise degree of hazard associated with air-borne lead of various size ranges and composition appears to be uncertain, but the particularly toxic nature of lead fume seems to be generally accepted. The importance of distinguishing between particle sizes has long been recognised by industrial hygienists, and it is plainly of advantage to know the lead fume content as distinct from the total lead content of an air-borne sample.[6 to 14]

One of the drawbacks of the usual chemical methods of determining "lead fume" is that the whole of the lead in the filter medium is determined irrespective of particle size. The presence on the filter of a few adventitious large particles of lead dust, which might themselves be of relatively low toxicity, could therefore result in an analytical result far greater than the true lead fume concentration. The method of developing the stain in the proposed field test permits this error to be avoided and the true lead fume content to be ascertained. It therefore has the advantage of determining with reasonable specificity a type of particulate lead of well defined origin and size range, which also happens to be a particularly toxic form.

When the test paper containing the collected sample is sprayed with acetic acid - acetone mixture, the particles of lead fume dissolve and combine with the tetrahydroxy-*p*-benzoquinone

already on the paper to form a uniform background stain. If, however, particles of lead oxide having a diameter greater than 10 μ are also present, these appear as deeply coloured pin-point stains. Particles of lead metal of the same size are less readily soluble and form coloured spots of lesser intensity, which, however, deepen in colour when spraying is repeated. The uniform background colour can usually be readily compared with the standard colour chart by ignoring any isolated pin-points of deeper colour. The result from the chart is taken as being the true lead fume content of the air.

Method

Apparatus—

Pump—A hand-operated or electric pump, which is capable of withdrawing a 15-litre sample against a pressure of 3·5 cm of mercury per litre per minute.

Test-paper holder—A suitable form of holder has been described.[3] The paper is placed between two polythene washers, which are held in position by two parallel brass plates carrying nuts and bolts. Plates and washers are drilled to take a hole of 1 cm diameter, and the sampled air passes through the paper by way of brass inlet and outlet tubes.

Insufflator—An all-glass spray type (manufactured by Messrs. Matburn Ltd., 25, Red Lion Street, London, W.C.1, for use in chromatography) fitted with a rubber bulb and reservoir is suitable.

Test papers—Strips of filter-paper 2½ inches × 1 inch.

Reagents—

*Tetrahydroxy-*p*-benzoquinone solution*—A 0·2 per cent. w/v solution in ethanol or methanol will retain its activity for several months, if kept out of contact with air. The extinction coefficient of a suitably diluted ethanolic solution of the crystals (obtained from Messrs. Purkis, Williams, 60, Brewery Road, London, N.7) at 310 mμ should not be less than 15,000.

Spraying liquid—Mix 1 volume of analytical-reagent grade lead-free glacial acetic acid with 3 volumes of analytical-reagent grade acetone.

Procedure—

Dip the strips of test paper in the tetrahydroxy-*p*-benzoquinone solution to a depth of 1 inch, drain, and allow to dry. Do not allow the fingers to come into contact with the impregnated portion of the test paper. Use papers so prepared the same day, and keep them out of contact with alkaline vapours.

By means of a hand-operated or electric pump, draw 15 litres of air through the holder containing the prepared test paper. If an electric pump is used, a reservoir to smooth out pulsations must be attached to the other end of the pump, and then a flowmeter. A variable by-pass on the electric pump is adjusted to maintain a flow rate of 3 to 5 litres of air per minute, which is indicated on the flowmeter.

Remove the test paper from the holder, and place the exposed side of the paper in a spray from the insufflator at a distance of 6 inches for 5 seconds. A uniform purple background stain appears within a few seconds, possibly accompanied by a few isolated pin-points of deeper colour. At the end of 1 minute, compare the background colour with the standard stain chart to ascertain the lead fume content. To detect or confirm the presence of large particles of lead dust, spray a second or third time to develop pin-points of deeper colour. Do not allow the test paper to become perceptibly damp.

Preparation of standard stains—

For the preparation of standard stains, a source of lead fume particles uniformly suspended in a fluid medium must be used. Larger particles, *e.g.*, of lead oxide or lead salts prepared by other methods, would not form a suspension stable enough for this purpose. A series of samples of increasing volume from a freshly prepared lead fume was collected on large circles of prepared test paper. The papers were sprayed to develop the stain, and the lead content on a measured portion of each paper was determined by the dithizone method. The amount of lead present on a 1-cm diameter circle of paper for each stain was then calculated. A series of standard stains representing 0·05, 0·1, 0·2, 0·4 and 0·8 mg of lead fume (as Pb) per cubic metre of air for a 15-litre sample is suitable. As the tint of the stains is not permanent, the freshly made stains were copied in water colours for reference purposes and for reproduction in printers' colours.

In a less accurate but less troublesome method, use is made of the ability of acetone to form a uniform suspension with lead fume particles. Lead fume is slightly soluble in acetone, and a stock solution of acetone saturated with lead fume must first be prepared. Lead fume was formed as previously described, drawn through a 6-inch head of water and collected on filter-paper in the holder. A mixture of pure redistilled acetone and not less than 5 mg per litre of lead fume was repeatedly shaken for 24 hours and then filtered through glass-fibre paper. This prepared acetone was used to make a suspension of lead fume containing 0·01 per cent. w/v of lead, from which a working standard suspension having one-tenth of this concentration was prepared. Portions of the standard suspension (0·15, 0·3, 0·6 and 1·2 ml) were filtered through pieces of glass-fibre paper held in the test-paper holder, the papers being previously wetted with prepared acetone to prevent lateral diffusion of the samples. The papers were removed, dried and sprayed successively with tetrahydroxy-*p*-benzoquinone solution and acetic acid - acetone mixture to develop standard stains of 0·1, 0·2, 0·4 and 0·8 mg of lead fume (as Pb) per cubic metre of air for a 15-litre sample.

Results

Samples of artificially prepared lead fume were collected on 10-cm diameter test papers. The stains were developed and compared with the standard colour chart by several observers. Separate portions of the papers (of known area) were then analysed by the dithizone method (by which, of course, the field method had originally been standardised) and by spectrographic examination. The results are shown in Table I.

Table I

Comparison between field, dithizone and spectrographic methods

Sample No.	Amount of lead found per sq. cm of test paper by field method, μg	Average amount of lead found per sq. cm of test paper by field method, μg	Amount of lead found per sq. cm of test paper by dithizone method, μg	Amount of lead found per sq. cm of test paper by spectrographic method, μg
1	1½, 1½	1·5	2·2	2·1
2	3, 3 to 4, 3, 3	3·0	2·9	3·2
3	4, 5	4·5	5·4	4·9
4	6½, 7, 7, 7, 7	7·0	7·3	7·6
5	9, 9, 8, 9	9·0	10·0	9·8
6	10, 10, 10½, 10 to 11	10·0	11·2	11·5

Discussion of the method

Sensitivity—

The method is sensitive to about 0·05 mg of lead per cubic metre; this figure may be somewhat higher if the atmosphere contains much carbonaceous matter. Above about five times the threshold toxic limit (0·2 mg of lead per cubic metre), the determination ceases to be precise. Air-borne particles of lead oxide or lead of the same size range as lead fume, which have not been formed by condensation, can also be determined by this method.

Interference by fumes of other metals—

Of the common metal fumes, those of cadmium and zinc also form coloured complexes with tetrahydroxy-*p*-benzoquinone under the conditions of the field test. Antimony fume may form a colour if more than the normal amount of spraying is carried out. The stains of these metals, when present in not more than moderate amounts, can be removed. Stains caused by cadmium and zinc can be removed by immersing the developed test paper in 10 per cent. v/v aqueous acetic acid for 30 seconds, washing with distilled water slightly acidified with acetic acid and drying with absorbent paper. An antimony stain can be similarly removed, together with any cadmium or zinc, by using a 5 per cent. aqueous solution of sodium hydrogen tartrate instead of dilute acetic acid; when this treatment is carried out, the tint of the residual lead stain is slightly altered. After removal of the stain of the interfering metal, the uncontaminated lead stain is compared with the standard stain chart in the usual way.

This work was carried out on behalf of the Committee on Tests for Toxic Substances in Air, and the Ministry of Labour and National Service.

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Applications of Infra-red Spectroscopy: The Identification and Determination of Gas-chromatographic Fractions

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The desirability of identifying products by a method other than comparison of retention times is stressed. Details of a simple method of collecting products separated from mixtures by gas chromatography are given, and the technique subsequently used for their identification and quantitative determination by vapour-phase infra-red spectroscopy is described. The many advantages of infra-red examination of vapours rather than liquids, when only fractional milligram amounts are available, are outlined. Amounts of about 5 μmole of all vapours or liquids having a boiling-point of up to about 175° C can be identified and quantitatively determined to within ±2 per cent. without difficulty. Experiments are in progress to increase the sensitivity and applicability of the method.

In routine analyses of products from well characterised reaction systems, and possibly for research investigations in certain fields,[1] it is often possible to obtain quantitative and qualitative results of acceptable accuracy from relative retention times on a carefully controlled column. For many purposes, however, identification of separated components on gas-chromatographic evidence alone is inadequate.

Column-retention times are purely relative and not always exactly reproducible; even when the chemical class (*e.g.*, ketone, hydrocarbon etc.) of the expected reaction products is known, identification by comparison of the retention time of the unknown with those of standards requires exact reproduction of column operating conditions.[2]

Difficulty can also arise when non-symmetrical peaks are produced[3]; the peak-maximum retention time of a component then depends on concentration. Peaks can also overlap,[4,5] and certain combinations of substances are often difficult to separate.[5] Chromatographically, a single peak is no criterion of purity, since more than one substance may be present[6]; the components present could often be resolved if their presence was suspected and alternative column operating conditions were selected.

Confirmation of homogeneity of fractions is therefore required, together with unequivocal identification and accurate quantitative determination of the product. The analytical method used should involve some property of the molecule other than boiling-point. Often,

only fractional milligram amounts of material can be recovered from a column; few methods therefore remain applicable. Mass-spectroscopic examination of fractions on this scale has been described,[4,7,8,9] but instances have been reported[10] in which infra-red spectroscopy was also required.

The tremendously powerful technique of coupling gas-chromatographic separation with subsequent infra-red identification has long been recognised and advocated.[11,12,13,14,15] Greater use of the combined techniques has not so far been made because samples for gas chromatography are normally smaller by a factor of about 10^2 than are those required for spectroscopy. However, attention is now being given commercially to production of infra-red cells and accessories for micro-analysis; for instance, a liquid cell of capacity 80 μl has been described.[16] Progress is also being made with scale-expansion techniques and development of infra-red microscopes, by which single fibres and crystals may be examined.

This paper describes a technique that has been used in this department during the past 2 years for, *e.g.*, the identification of gas-chromatographically fractionated products from the following investigations—

(*i*) Reactions of methylene radicles with propylene and *cyclo*propane,[17] *iso*butene[18] and acetylenes and allenes.[19]
(*ii*) High-temperature oxidation of propane.[20]
(*iii*) Photolysis of *cyclo*pentanone.[21]
(*iv*) Irradiation of organic chloro compounds with cobalt-60.[22]
(*v*) Retention of solvents and moisture by carbohydrates.[23]
(*vi*) Acidic decomposition products of carbohydrates.[24]

Gas - liquid chromatography has been used throughout for separation, but the technique is applicable to products separated by gas - solid chromatography. In addition, it has been used for the collection and infra-red examination (without prior separation by gas - liquid chromatography) of mixtures of reaction products, such as methyl and ethyl iodides from semi-micro alkoxyl determinations[25] and carbon dioxide and furan from uronic acid determinations.[26]

The method has the advantages of (*a*) ease of handling, particularly of volatile liquids, (*b*) greatly increased sensitivity, (*c*) increased accuracy of quantitative determination, and (*d*) ease of quantitative recovery of all samples. No originality is claimed for the technique; it is described in the belief that its simplicity, scope and advantages may not be fully recognised. No expensive ancillary infra-red equipment is required.

Experimental

Apparatus and procedure—

Fig. 1 (*b*) shows the dimensions of the trap used. The exit of the gas-chromatographic column is connected (via an anhydrone tube if necessary) by a B10 ground-glass joint to A (traps made more recently have hemispherical joints). With an anhydrone guard-tube fitted to B, trap XY is immersed in a flask of liquid nitrogen. Any carrier gas can be used, although carbon dioxide, if used, must be removed before passage through the trap. At flow rates of 15 to 20 ml per minute, milligram amounts of vapours and liquids having boiling-points greater than about −120° C are quantitatively trapped with no retention of carrier gas. Under these conditions, the trap appears to have an adequate safety factor, and discrete frozen droplets, which could be swept as such through the trap, do not form.[27] Should, however, this simple trap prove to be inadequate under certain conditions, a trap for recovery of extremely small fractions has been described.[28] A simple 3-way stopcock junction system similar to that already described[5] has been used to facilitate collection, in separate traps, of fractions emerging from a column in close succession.

The trapped samples, stoppered at A and with tap T_1 closed, are kept in liquid nitrogen until required. The B10 cone of an evacuated gas-cell is inserted into A, shown in Fig 1 (*b*), tap T_2 being closed. The cone B is then attached to a vacuum-line, with trap XY still immersed in liquid nitrogen. Taps T_1 and T_2 are then opened and the whole system is evacuated to a pressure of between 0·5 and 1 mm of mercury. Tap T_1 is then closed, the assembly is removed from the vacuum-line and an anhydrone guard-tube is fitted to B. Trap XY is then withdrawn from the liquid nitrogen and immersed in boiling water or warmed gently in a bunsen flame. The former method of heating is preferred, but the latter was found to be necessary for higher-boiling liquids. When the trapped substance has completely

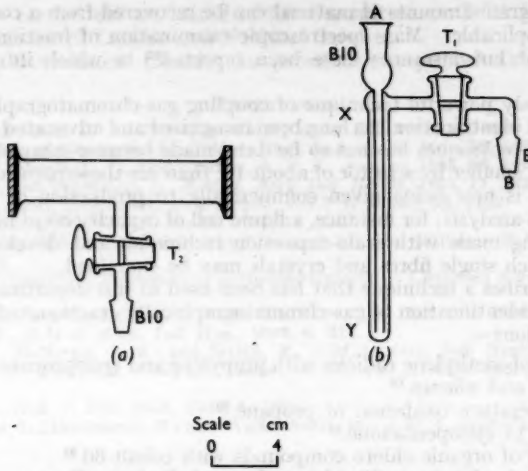

Fig. 1. Apparatus used for collecting separated products: (*a*) gas-cell, (*b*) trap

vaporised, tap T_1 is quickly opened so that the residual vapour in trap XY is swept into the gas-cell; the contents are then at atmospheric pressure. This serves as a standard condition for the examination; daily variations in atmospheric pressure are insignificant and the conditions for automatic double-beam compensation for atmospheric carbon dioxide and water vapour are not upset. The procedure also gives increased sensitivity; when the total pressure is made atmospheric by adding infra-red inactive gases to a gas-cell containing a compound at a partial pressure of 1 mm, the peak height is greater than that produced when the total pressure is allowed to remain at 1 mm. "Pressure broadening" effects are considered later.

RECOVERY OF SAMPLES—

Replace the cone of the gas-cell in socket A, and attach B to the vacuum-line. With tap T_2 closed, evacuate trap XY and immerse it in liquid nitrogen. Close tap T_1 and open tap T_2. Close tap T_2, and, after 10 to 20 seconds, open tap T_1 and re-evacuate trap XY. Repeat this cycle several times, then finally evacuate completely with taps T_1 and T_2 both open. Quantitative recovery has been repeatedly proved by returning the recovered product to the gas-cell in the usual way and re-running the spectrogram; the peak heights previously obtained are duplicated. When it is found that insufficient material has been available for analysis, recovery of the sample into the trap and collection therein of the identical peak from a second chromatographic separation generally permits sufficient material to be collected. No undesirable retention of products in the traps or gas-cells has been observed if continuous evacuation for 10 to 15 minutes is carried out between analyses. The amount of Apiezon or silicone grease on taps and ground-glass joints should, however, be kept to a minimum.

DISCUSSION OF THE METHOD

APPLICABILITY—

When conventional infra-red unheated gas-cells fitted with sodium chloride end-plates secured by Bedacryl 122X resin (kindly supplied by Imperial Chemical Industries Ltd., Dyestuffs Division) are used, the method is applicable to all vapours and liquids of low or medium boiling-point. Generally, partial pressures of 1 to 2 mm admitted to a cell of volume 64 ml (the pressure subsequently being made atmospheric in the way already described) give satisfactory spectra. No condensation of the substance occurs during the period of examination (usually 10 to 15 minutes) if the boiling-point is not too high. Spectra for furfuryl alcohol (boiling-point 170° C) and for benzaldehyde (boiling-point 179° C) have been obtained in this way, but boiling-points of 175° to 180° C probably represent the upper limit for unheated cells. The use of heated cells is well known,[29,30] however, and experiments are in progress to discover how far their use will extend the applicability of this technique.

SENSITIVITY—

The infra-red absorption of different compounds varies widely, and the method has maximum sensitivity for compounds (*a*) of a polar character, and (*b*) of low molecular weight. The data in Table I, however, are representative; for predominantly non-polar compounds, the sensitivity will only decrease by a factor of about 2. When a gas-cell of length approximately 12·5 cm, internal diameter approximately 2·5 cm and measured internal volume 64 ml is used, the sensitivity shown in Table I is attainable.

TABLE I

SENSITIVITY OF THE METHOD

Compound	Minimum amount of compound detectable, μg	Minimum amount of compound detectable, μmole	Partial pressure required	
			Minimum, mm of mercury	Maximum, mm of mercury
Methanol	130			
Ethanol	190			
Acetone	240			
*iso*Propyl alcohol	250	4 to 5	1·0	2·0
Diethyl ether	300			
Chlorinated solvents	—			
Benzene	470			
Furan	410	6 to 7	1·5	2·5
Dioxan	530			

Use of the partial pressures shown in Table I gives satisfactory spectra and permits positive identification of the compound; possible information regarding freedom from contaminants is also provided. In certain limited instances, when the reaction product is almost certainly known, confirmation of identity and an estimate of the amount present can be made on much smaller amounts (about 10 per cent. of those in Table I) by referring only to the most intense absorptions in the spectrum.

QUANTITATIVE DETERMINATIONS—

Spectra for the liquid and vapour forms of a compound usually differ. Identification must therefore be made by comparison with spectra for standard vapours. A series of standard spectra can be obtained over as wide a concentration range as is desired for each compound under investigation by using a simple manometer system, the essential features of which are shown in Fig. 2. Initially, a partial pressure of the vapour under test equal to 16 mm of mercury is admitted to the 64-ml gas-cell, this pressure being read from the scale (graduated in millimetres) attached to the manometer limb. With care, satisfactory

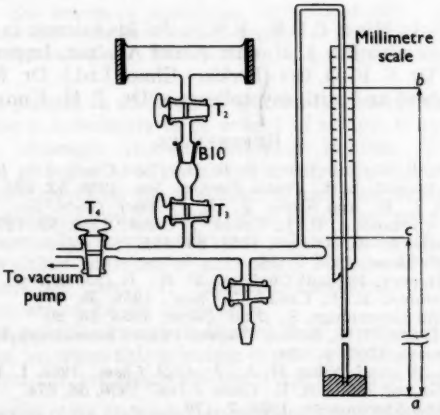

Fig. 2. Manometer system used for determinations

reproducibility in this operation was possible. Accurate dilutions in the gas-cell, which, in turn, give partial pressures of 8, 4, 2, 1 and 0·5 mm of mercury, are subsequently conveniently made by using the manometer, which can readily be constructed by successive glass-working refinements so that its internal volume, when a pressure equal to 50 per cent. of atmospheric is being indicated, is equal to that of the gas-cell. This is easily checked experimentally. The gas-cell, with contents at atmospheric pressure and tap T_2 closed, is fitted to the manifold, as shown in Fig. 2. The manifold system is completely evacuated with tap T_3 open; mercury rises to level *b*. The correct internal volume of the manifold is attained when opening tap T_2 causes the mercury level to drop from *b* to *c*, where $ac = ab/2$. Construction of a manifold satisfying this condition to within 3 mm of mercury was easily achieved; this was considered to be adequate, as daily variations in atmospheric pressure were not taken into account.

When the partial pressure of the gas under examination has been halved in this way, air is admitted to restore atmospheric pressure in the gas-cell; the infra-red spectrum for this concentration is then recorded.

The method described is considered to permit determination of concentrations to within ± 2 per cent. Standardisation need not be tedious, since, for any vapour under test, calibration on any one carefully selected characteristic absorption in the spectrum is usually adequate. Peak areas, not peak heights, are proportional to concentration.[31,32] The relationship between peak height and concentration is not linear, and care should be taken to compare peak areas when comparisons are made with spectra representing standard concentrations.

Obvious refinements to the apparatus can be introduced should increased accuracy of determination be desired. Consideration would then have to be given to the effect of "pressure broadening"; in a gaseous mixture, each gas (even homopolar diatomic gases that have no infra-red absorption) affects the optical density of the others, thereby causing deviations from Beer's law. Calibration curves for correcting these deviations can be experimentally plotted and applied, so permitting concentrations to be measured to within $\pm 0{\cdot}1$ per cent.[33]

DETAILS OF INFRA-RED SPECTROMETER—

A Hilger H800 double-beam instrument was used; it has a large area available for the introduction of sample cells and can accommodate cells up to 60 cm in length. Experiments are in progress to evaulate the ultimate sensitivity of this method without recourse to the use of optical cells of multiple path length.[34,35] For instance, recent work has shown that a gas-cell (length 32 cm, internal diameter 1·5 cm and volume 51 ml) constructed by fusing together the non-flange ends of two ground-glass flanges (Quickfit and Quartz type FG15) increases the sensitivity shown in Table I by a factor of 40 to 50. The use of a gas-cell of this length introduces appreciable energy losses in the sample beam (an effect not caused by a 12·5-cm cell) and it is necessary to insert a compensating cell of similar dimensions in the reference beam. Further work is in progress, however, and will be reported elsewhere at an early date.

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The Volumetric Determination of Chloride in Titanium and Zirconium by a Polarisation End-point Procedure*

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The development of an amperometric procedure is described. The recommended method is directly applicable to acid solutions containing either titanium or zirconium. It is simple, rapid and almost entirely free from personal bias; the necessary apparatus costs about £50. A modified form of the procedure has been successfully applied to the determination of silver in certain non-ferrous alloys; further work is in progress.

The amount of chloride present in all forms of titanium and zirconium rarely exceeds 0·1 per cent., and, provided that a sufficiently large weight of sample is available, the conventional gravimetric procedure, although time-consuming, is reliable. Turbidimetric procedures, based on the formation of colloidal silver chloride, have the advantage of being rapid and directly applicable,[1] but depend on the preparation and comparison of solutions that must be prepared under rigorously controlled conditions if results are not to be erratic. Volumetric procedures, although normally direct and rapid, are not generally applicable to the determination of chloride in either titanium or zirconium, because of end-point difficulties when dilute standard solutions are used. It appeared, therefore, that an electrical means of detecting the end-point would be an advantage.

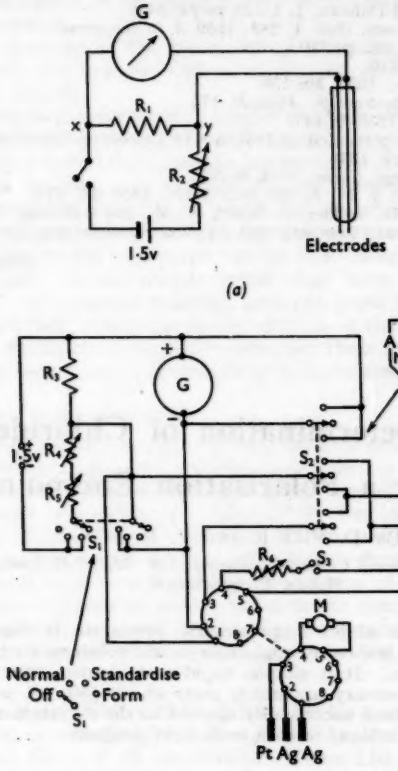
An examination of published work indicated that a polarisation end-point procedure[2] had distinct possibilities, as, when this principle is used, titrations can be made with solutions as dilute as 0·001 *N* and the necessary electrical apparatus is simple to construct.

* Presented at the meeting of the Midlands Section of the Society at Birmingham on Tuesday, September 16th, 1958.

In such procedures, a small e.m.f. is applied across two similar electrodes immersed in a stirred solution, and the current change during the titration is followed on a galvanometer. The main types of titration are—

(*i*) those that involve only one reversible electrode process, the end-point being shown by the current falling to or rising from a small residual value,[3] and

(*ii*) those that involve two different reversible electrode processes. In this type, both reagents react reversibly at the electrodes, and the current falls to a minimum at the end-point of the titration and rises when excess of titrant is added.



R_1, R_3 = 200-ohm resistor
R_2 = 30,000-ohm variable resistor
R_4 = 25,000-ohm variable resistor (standardising-control coarse adjustment)
R_5 = 5000-ohm variable resistor (standardising-control fine adjustment)
R_6 = 50-ohm variable resistor (stirrer motor speed control)
S_1 = Main switch
S_2 = Form switch
S_3 = Stirrer switch
G = Galvanometer, Pye Scalamp, type 7893/S
M = Stirrer motor, Ever Ready, type TG18E

Fig. 1. Circuit diagram of titration apparatus: (*a*) potentiometer circuit; (*b*) complete circuit

In the original "dead-stop end-point" method of Foulk and Bawden,[4] two platinum electrodes were used to detect the end-point in titrations of iodine against thiosulphate. Later, Clippinger and Foulk[5] attempted to extend this method to precipitation titrations, but the end-point was sluggish, especially in acid solutions.

Subsequently, Bradbury[6] proposed the use of two identical silver - silver chloride electrodes for argentimetric titrations. As both silver ions and chloride ions form reversible systems at a silver - silver chloride electrode, the titration corresponds to type (*ii*) above. Independently, Samson[7,8] claimed to have used similar electrodes for this titration, but he used a complicated circuit and gave no details of its analytical application.

The object of this investigation was the development of a suitable second-order electrode pair, its incorporation in a "dead-stop" circuit and its application to the determination of chloride in titanium and zirconium.

Experimental

The potentiometer circuit used is shown in Fig. 1 (*a*) and was similar to that described by Freedman.[9] The galvanometer was a Pye Scalamp moving-coil model with a critical damping resistance of 10,000 ohms and a maximum sensitivity of 200 mm per μA; it was shunted to provide four sensitivity ranges. In order to standardise the conditions under which titrations were made, the electrodes were short-circuited and the variable resistor was adjusted to produce a defined galvanometer deflection. By calibrating the galvanometer in millimetres per microvolt, the deflection was related to the voltage measured across xy; all quoted applied voltages (V_{xy}) refer to measurements made in this way. Standardisation was effected before each titration to ensure constant and reproducible circuit conditions.

A pair of identical second-order electrodes, consisting of two silver wires coated with silver chloride, was substituted for the normal platinum electrodes, and 20 ml of 0·01 *N* sodium chloride were titrated with 0·1 *N* silver nitrate. The end-point of the titration was indicated by a well defined minimum in the curve (see Fig. 2, curve A), and this system was therefore chosen as a basis for further investigation.

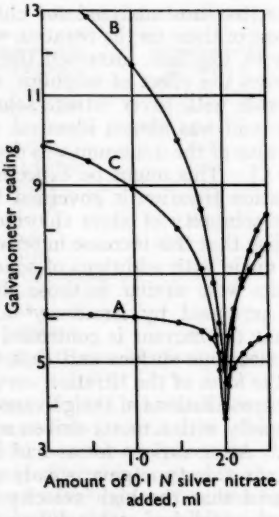


Fig. 2. Effect of acids on the titration curve. The titration of 20 ml of 0·01 *N* sodium chloride was carried out in a total volume of 100 ml, the applied voltage was 100 mV and the galvanometer setting was 0·01: curve A, neutral solution; curve B, solution 0·1 *N* in sulphuric acid; curve C, solution 0·1 *N* in acetic acid

DEVELOPMENT OF A SUITABLE ELECTRODE PAIR—

Electrodes should be robust, easily prepared and capable of reproducible performance, particularly when the procedure is intended for routine application. Two silver wires were sheathed in polythene, 1 cm of each being allowed to protrude, as shown in Fig. 1(*a*). The silver chloride layer was prepared by electrolysing potassium chloride solution with the two silver wires as anode and a piece of bright platinum wire as cathode. (This is subsequently referred to as "plating".)

Although the first electrode pair proved to be satisfactory, it was necessary to establish whether or not other similarly prepared electrodes would be reliable. Titrations in which electrodes prepared with slight variations in the plating conditions were used proved that the position of the end-point was unaffected, but the sharpness of the minimum, *i.e.*, the sensitivity of the titration, was altered. Experiments were made to show how sensitivity varied with the method of preparing the electrode pair.

It was established that plating at 3 volts produced a spongy uneven deposit, and, at 1-volt or less, plating was too sluggish. Rapid plating and a smooth adherent deposit were achieved at an applied voltage of 1·5 volts, and this potential was used subsequently.

Electrodes were prepared by plating for different lengths of time. Sensitivity was greatest after short plating times, which correspond to the formation of thin chloride layers, but, in practice, such electrodes deteriorated and showed patches of bare metal. To compromise between sensitivity and electrode stability, a plating time of 5 minutes was adopted. In all these experiments, the polarity of the cell was reversed for a few seconds in the first minute of plating[8] to improve the adherence of the deposit. The procedure finally adopted for preparing the electrode pair is described under "Method."

The electrodes were stored in water and no special precautions were taken to exclude light during their life, nor were any necessary. One pair of electrodes showed no loss in sensitivity after fifty titrations and there was no difference in sensitivity when one electrode was made the anode after it had been used as the cathode in twelve titrations. The relative positions of the two electrodes did not affect the form of the titration curve; this was shown by making the same titration with the electrodes inclined at various angles.

TITRATION CHARACTERISTICS—

Added electrolyte—Solutions that are analysed for chloride frequently contain anions other than chloride, and the effects of these on the titration were examined. Sulphuric, nitric and acetic acids, present singly or together, increased the sensitivity, the end-point being unaltered. Fig. 2, curve B, shows the effect of sulphuric acid present during the titration of 20 ml of 0·01 *N* sodium chloride with silver nitrate solution of approximately the same normality. The effect of nitric acid was almost identical with that of sulphuric acid. In the presence of acetic acid, the value of the minimum was decreased and there was an increase in sensitivity (see Fig. 2, curve C). This might be expected, as Bradbury[6] stated that the end-point current in a precipitation titration is governed by the solubility product of the precipitate and also because the solubility of silver chloride is decreased in the presence of acetic acid.[10] In order to establish that this increase in sensitivity was not merely a function of pH, separate titrations were made with additions of solutions of potassium sulphate and nitrate; in both instances, results were similar to those shown by curve B, Fig. 2.

Rate of stirring—Theories proposed by Bradbury[6] and by Samson[8] on "dead-stop end-point" titrations suggest that the current is controlled by the rate of diffusion of ions across the double layer at the electrode surface and that the rate of stirring the solution might be expected to influence the form of the titration curve. In preliminary experiments, magnetic stirring produced erratic oscillations of the galvanometer spot. This was overcome when the solution was stirred rapidly with a motor-driven screw-shaped paddle, but solution was lost by excessive splashing. After various forms had been tried, a paddle shaped like an inverted T was finally used, as this type gave steady galvanometer readings at much slower motor speeds. It appeared that the high velocity of the solutions relative to the electrodes avoided turbulence and established stable diffusion conditions in the region of the electrodes.

Applied voltage—The choice of applied voltage depends on the sensitivity of the electrode pair, the galvanometer sensitivity and the amount of chloride present in solution. A series of titrations was carried out with the same electrode pair at a chloride concentration of 0·71 mg per 100 ml, but different voltages were applied.

A large applied e.m.f. produces a large galvanometer deflection, but, at voltages in excess of 60 mV, this is so large that a lower galvanometer sensitivity must be used and the form of the titration curve deteriorates. Further, an increase in the amount of chloride present has the same effect, *i.e.*, at 60 mV the current produced by amounts of chloride in excess of 0·71 mg per 100 ml is too large to measure, and, in order to retain the advantage of high galvanometer sensitivity over the range of chloride concentration normally encountered, an applied e.m.f. of 30 mV was chosen. This value was satisfactory for most purposes. For the detection of amounts of chloride in the region of 0·05 mg per 100 ml, the applied e.m.f. may be increased to 100 mV; this retains a galvanometer sensitivity of 0·1.

DETERMINATION OF CHLORIDE IN TITANIUM—

Solutions containing chloride (at the level normal for titanium) in the presence of sulphuric acid and various added amounts of titanium solution were titrated with standard silver nitrate solution. The resulting curves showed that even a 200-fold excess of titanium ions over chloride ions had no effect on the position of the end-point minimum.

The direct determination of chloride added to 1-g portions of commercially pure titanium (dissolved by using sulphuric and fluoroboric acids[11]) was investigated. Dilution of the sample solution served only to impair the sharpness of the end-point. All chloride originally added was recovered, and the presence of a small excess of about 0·05 mg (equivalent to 0·005 per cent. of chloride in the commercially pure titanium) was indicated, a value almost identical with that determined turbidimetrically.[1] Amperometric determinations were made on several miscellaneous samples of titanium sponge and results were compared with those found by using the turbidimetric procedure. These, together with some gravimetric results, are shown in Table I. To minimise the effect of segregation in the examination of the Japanese titanium sponge, a 10-g sample was used and determinations were made on aliquots (representing 1-g samples) of the resulting solution. In this way titrations were shown to be reproducible.

TABLE I

DETERMINATION OF CHLORIDE IN TITANIUM

Sample No.	Amount of chloride found by— amperometric procedure, %	turbidimetric procedure, %	gravimetric procedure, %
1	0·062, 0·062	0·04	—
2	0·055	0·04	—
3	0·055	0·05	—
4	0·090, 0·089	0·07	—
5	0·084	0·08	—
6	0·088	0·08	—
7*	0·11	0·09	—
8*	0·10	—	0·12
9*	0·13	—	0·12, 0·135
10*	0·062, 0·062, 0·064	—	0·066
11*	0·048, 0·048, 0·050	—	0·050, 0·047
12*	0·045	—	0·049
13*	0·083	0·08	—

* Samples of Japanese titanium sponge.

In addition to the results shown in Table I, nineteen samples of titanium metal (previously examined by the turbidimetric procedure in a control laboratory and reported to contain less than 0·01 per cent. of chloride) were re-examined by the same analyst, the proposed amperometric procedure being used. The results were in the range 0·002 to 0·011 per cent. of chloride, with a precision of about 0·002 per cent. at the higher concentration.

Solutions containing 1 g of commercially pure titanium (0·005 per cent. of chloride) and various added amounts of chloride were examined by the amperometric procedure. Titration curves (see Fig. 3) show that the method can be successfully applied over a wide range of chloride content. Additions of up to 0·5 per cent. of both iron and magnesium, which represent excessive amounts of these impurities, do not affect the determination or the form of the titration curve.

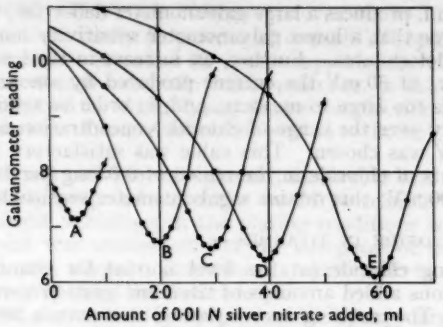


Fig. 3. Determination of chloride added to solutions of titanium: curve A, 0·017 per cent.; curve B, 0·071 per cent.; curve C, 0·106 per cent.; curve D, 0·14 per cent.; curve E, 0·21 per cent.

When the procedure described under "Method" is used, the chloride content of a sample of titanium can be determined within 5 minutes of dissolution.

During development work, titration curves were plotted for each sample, but this would not be necessary in routine practice. Because the galvanometer response is rapid, the titrant can be added rapidly at the beginning of the titration, but, near the end-point, the solution must be added dropwise and the position of the galvanometer spot moving across the scale must be noted. At the end-point, the spot reaches a minimum, and the first excess of silver ions causes an increase in current; the end-point can be detected to within 0·05 ml of 0·01 *N* silver nitrate.

DETERMINATION OF CHLORIDE IN ZIRCONIUM—

The proposed procedure was used in the assay of a sample of zirconium sponge; the results, which compare favourably with those found by a turbidimetric procedure similar to that used for determining chloride in titanium, were as follows—

Amount of chloride found by amperometric procedure, % ..	0·039	0·039	0·039
Amount of chloride found by turbidimetric procedure, % ..	0·030	0·035	0·035

The chloride content of zirconium metal is considerably lower than that of zirconium sponge, and, to determine this amount precisely, it was necessary to reduce the volume of solution used in the final titration and to titrate with a micrometer-syringe burette. Details of this modification are described under "Procedure." This modification was used in the determination of amounts of chloride, as sodium chloride, added to 1-g samples of zirconium metal containing 0·0005 per cent. of chloride. The results were as follows—

Amount of chloride added, %	0·0010	0·0020	0·0030	0·0050
Amount of chloride found, %	0·0008	0·0018	0·0032	0·0047

Comparative figures by an alternative procedure are not available, as neither the turbidimetric nor the gravimetric procedure gives reliable results at this level.

DETERMINATION OF SILVER—

The general form of the titration curve suggested the possibility of titrating silver ions in solution against standard sodium chloride solution. Pure solutions of silver containing 2 mg of silver per 100 ml were titrated successfully, curves being identical with those for the reverse titration. Subsequently, silver was determined amperometrically in several alloys containing large amounts of zinc, copper, cadmium and aluminium. Further work is in progress.

METHOD

REAGENTS—

Nitric acid, sp.gr. 1·42.

Sulphuric acid, diluted (1 + 3) *and* (1 + 4).

Fluroboric acid—To 280 ml of hydrofluoric acid maintained at 10° C add, in small amounts, 130 g of boric acid. Store the solution in a polythene bottle.

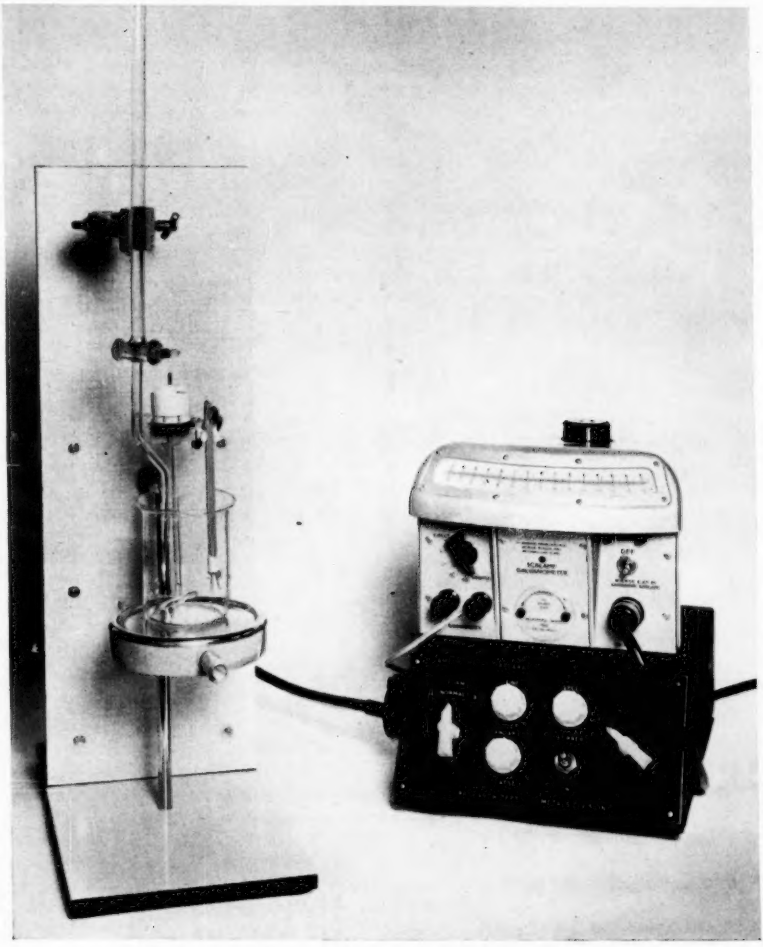


Fig. 4. Apparatus for determining chloride in titanium

[To face p. 60.

Potassium chloride solution, 2 per cent. w/v.

Sodium chloride solution, 0·01 N—Prepare by diluting a 0·1 *N* stock solution.

Silver nitrate solution, 0·01 N—Prepare by diluting a 0·1 *N* stock solution. Standardise against 0·01 *N* sodium chloride, as described under "Procedure."

1 ml ≡ 0·355 mg of chloride.

CONSTRUCTION OF ELECTRODE—

Solder a 1-inch length of 1-mm diameter platinum wire to a piece of bare copper wire, and sheathe both with 1·5-mm diameter polythene tubing. Sheathe two 6-inch lengths of 0·5-mm diameter silver wire with 1-mm diameter polythene tubing. Enclose the three wires in a length of 5·0-mm diameter polythene tubing so that, at one end, about 0·5 inch of the two silver wires and 0·5 inch of platinum wire are left bare. At the other end, allow about 1 inch of bare wire to project (for connecting to the rest of the circuit). At the end from which the silver and platinum wires project, heat the polythene in a stream of hot nitrogen until it fuses around the wires and makes a watertight seal. Allow the polythene to cool and harden, then test the insulation between the three individual wires. Trim the two silver wires at the sealed end to the same length (about 1 cm), and remove any surface deposit by carefully scraping with a knife. Connect the completed electrode pair to the rest of the circuit by soldering the two silver wires to the leads, Ag, from the supply circuit and the copper wire from the platinum electrode to the lead, Pt (see Fig. 1).

FORMATION OF SILVER CHLORIDE LAYER—

Rinse the silver electrodes with ammonium hydroxide, sp.gr. 0·940, and wash them thoroughly with water. Place the electrodes in a static solution of 2 per cent. w/v potassium chloride, and move the main switch to FORM (see Fig. 4). Move the Form switch to position B, allow the electrolysis to proceed for 10 seconds, and then switch to position A for 5 seconds. Repeat these operations five times, and then complete the plating by electrolysis in position B for 5 minutes. Return the Form switch to NORMAL and the main switch to OFF, remove the electrodes from the solution, and rinse them well with water. The two silver electrodes should have a smooth dark-brown coating; store the electrodes in water after use and between determinations.

One pair of electrodes in daily use will last for several weeks. To re-plate the electrode pair, remove previous deposits by scraping, and, after washing in ammonium hydroxide, sp.gr. 0·940, proceed as described in the previous paragraph.

PROCEDURE—

Transfer 1 g of the sample to a 150-ml squat beaker (see Note), and dissolve it in 60 ml of diluted sulphuric acid (1 + 4) and 1·0 ml of fluoroboric acid; warm gently to assist dissolution. Maintain the level of solution by adding water. Oxidise with a slight excess of nitric acid, sp.gr. 1·42, added dropwise, and then heat the solution just to the boiling-point. Cool, and filter if necessary through a Whatman No. 40 filter-paper into another 150-ml beaker. Place the beaker on the apparatus, and start the stirrer. Move the main switch to NORMAL, and, at a galvanometer setting of 0·1, adjust the coarse and fine standardise controls to give a scale reading of about 12. Place the tip of the microburette beneath the surface of the solution in the beaker, and add 0·01 *N* silver nitrate. Titrate as rapidly as possible; near the end-point, note the galvanometer reading after each 0·1-ml addition of titrant. The point at which the galvanometer spot reverses its direction is the end-point. Calculate the chloride content (it is essential to determine a blank value for the reagents, especially when the chloride content of the sample is less than about 0·01 per cent.).

NOTE—If the sample is expected to contain less than 0·01 per cent. of chloride, transfer 1 g to a 100-ml conical flask, and dissolve it in 10 ml of diluted sulphuric acid (1 + 3) and 1·0 ml of fluoroboric acid. Proceed as described, filter, if necessary, into a 15-ml beaker, and titrate with 0·01 *N* silver nitrate, but add the titrant from a micrometer-syringe burette in 0·01-ml increments.

CONCLUSIONS

The proposed procedure permits direct determinations of chloride to be made accurately in less than 5 minutes after dissolution of the sample in conventional solvents. This compares with about 30 minutes by existing turbidimetric procedures, which are less reliable and must

be carried out under rigorously controlled conditions, and 24 hours by the existing gravimetric procedure, which is only applicable when the available weight of sample is sufficient to yield at least a few milligrams of silver chloride precipitate.

It has been shown that solutions containing 1 mg of chloride can be titrated to an accuracy within ± 1 per cent., and under the best possible titration conditions as little as 0·5 μg of chloride per ml can be determined with a reproducibility of better than 20 per cent.

The procedure has potentialities in the determination of small amounts of silver, and work in this direction is in progress.

We thank Mr. W. T. Elwell, Division Chief Analyst, for practical suggestions and assistance in the preparation of this paper.

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The Volumetric Determination of Chloride in Paper and Water by a Polarisation End-point Procedure*

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A method described by the authors for the volumetric determination of chloride in titanium and zirconium has, with slight modification, been successfully applied to the determination of chloride in paper, board, etc., and some types of water.

Specifications for chloride in paper, board and similar materials normally stipulate that chloride determinations must be made on an aqueous extract of the sample; the final solution usually contains about 1 mg of chloride per 100 ml.

The determination is often made volumetrically, a visual indicator and 0·01 *N* silver nitrate being used, but, when the extracts are highly coloured, a gravimetric procedure must be used, as turbidimetric procedures are difficult to apply. The gravimetric procedure is time-consuming and the accuracy of the determination is impaired by the small amount of silver chloride finally weighed.

A British Standard method is available for the determination of chloride in "Papers for Electrical Purposes."[1] The method is based on an electrometric argentimetric titration with a silver-wire indicator electrode and a silver - silver chloride end-point half-cell. This procedure is reliable and has distinct advantages over the gravimetric and turbidimetric procedures.

* Presented at the meeting of the Midlands Section of the Society at Birmingham on Tuesday, September 16th, 1958.

The procedure developed in our laboratories for the determination of chloride in titanium and zirconium[2] has been successfully applied to the determination of chloride in similar types of paper, and, in several respects, has advantages over that recommended in the British Standard. For example, in the British Standard method a graph of galvanometer readings against volume of titrant added must be plotted for each titration and the salt bridge used must be cleaned out and replenished after each test. In the proposed procedure, the end-point is determined by simple observation of a galvanometer spot and the apparatus is simple to operate, is robust and has universal application.

It has already been established that the sensitivity of the end-point is improved by the presence of free acid, particularly acetic acid, and, accordingly, aqueous extracts from typical samples of papers, etc., were acidified with 10 ml of glacial acetic acid before titration.

Experimental

Determination of chloride in papers—

Aqueous extracts of papers were prepared by boiling 20-g samples with water for 30 minutes. After filtration, each extract was diluted to 1 litre and a 100-ml portion was titrated. The results, which are shown in Table I, were consistent and reproducible; they compared favourably with those found by current procedures. Papers that gave coloured extracts were titrated successfully, and results showed that the extracted dye has no deleterious effect on the electrodes. In the absence of completely satisfactory procedures for the precise determination of the chloride content of papers, the relationship between chloride added and chloride recovered was taken as a criterion for the success of the amperometric titration. For all papers examined in this way the recovery was 100 per cent. (see Table II), and, in consideration of the results in Table I, results are considered to be more reliable than those that would have been found by the alternative procedures. Approximately 0·01 *N* silver nitrate, which was standardised daily against 0·01 *N* sodium chloride, was used in all determinations.

Table I

Determination of chloride in paper, cardboard and similar material

Sample	Amount of sodium chloride found by— amperometric procedure, %	gravimetric procedure, %	volumetric procedure, %
Cartridge paper*	0·026, 0·026, 0·027, 0·025, 0·025, 0·026	0·033, 0·036	—
	0·031, 0·032	0·033	—
	0·097	0·081	—
	0·036	0·046	—
	0·024, 0·023	0·018	—
	0·025, 0·024	0·016	—
	0·027, 0·027	0·018	—
Waxed paper	0·057, 0·057	0·070	0·06
	0·040	0·054	0·04
	0·036, 0·038	0·050	0·06
Cardboard	0·017, 0·018	—	0·032
Cotton	0·011, 0·012	—	0·035

* The routine volumetric procedure was not applicable, as the extracts were coloured.

Determination of chloride in water—

Successful application to the examination of aqueous extracts of paper suggested that the amperometric procedure might be applicable to the examination of boiler water, condensate and similar materials containing small amounts of chloride. Direct titration of an acidified solution was satisfactory for most samples, as shown by the results in Table III. Some samples, however, failed to yield the characteristic titration curve when silver nitrate solution was added and the turbidity caused by colloidal silver chloride was slow to appear. This behaviour might be due to the presence of organic matter in the water sample, but further investigations will be necessary before the procedure can be extended to the routine examination of all water samples irrespective of their origin and composition.

Table II

Recovery of added sodium chloride from samples of paper

The sodium chloride was added to the aqueous extracts of the samples

Sample No.	Amount of sodium chloride originally present found by— amperometric procedure, %	gravimetric procedure, %	Amount of sodium chloride added, %	Amount of added sodium chloride found by amperometric procedure, %
1	0·026, 0·027	0·037	0·023	0·023
2	0·031	0·035	0·023	0·022
			0·047	0·046
3	0·040	0·054	0·0047	0·0048
4	0·097	0·081	0·0047	0·0044
			0·023	0·024
5	0·036	0·046	0·012	0·012
			0·023	0·023
			0·047	0·048

Table III

Determination of chloride in water

Sample	Amount of chloride found by— amperometric procedure, p.p.m.	volumetric procedure, p.p.m.
Boiler water ..	31·9, 31·9	32
	234	231
	21·3	—
	1·4	2
	1·2	1
River water	170	168
Condensate	<0·2	<0·4
Tap-water	6·3, 6·3, 6·3	—

Method

The apparatus and reagents were as previously described[2] (see p. 55).

Procedure for papers and allied materials—

Cut 20 g of sample into pieces about 1-inch square, and extract by gently boiling with 300 ml of water for 30 minutes. Cool to room temperature, and decant the solution through a Whatman No. 541 filter-paper. Wash the filter-paper with water, adjust the volume to 500 ml, and mix. Carry out the determination on the extract as described below.

Procedure for aqueous extracts and water samples—

Transfer a 100-ml aliquot to a 150-ml tall beaker, add 10 ml of glacial acetic acid, place the beaker on the apparatus, and start the stirrer. Move the main switch to the NORMAL position, and, with a galvanometer setting of 0·1, adjust the coarse and fine standardise controls to give a scale reading of about 12. Place the tip of the microburette beneath the surface of the solution in the beaker, and add 0·01 *N* silver nitrate. Titrate as rapidly as possible; near the end-point, note the galvanometer reading after each 0·1-ml addition of titrant. The point at which the galvanometer spot reverses its direction is the end-point. Calculate the chloride content.

We thank Mr. W. T. Elwell, Division Chief Analyst, for practical suggestions and assistance in the preparation of this paper.

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Notes

DETERMINATION OF ACRYLONITRILE IN VARIOUS MATERIALS

OUR recently published method[1] for the detection of traces of acrylonitrile by paper chromatography (based on the reaction between acrylonitrile, thiourea and hydrochloric acid) may be applied to various materials. In practice, there are usually only trace amounts of acrylonitrile dispersed in a large excess of material that can interfere with the chromatographic procedure. Examples of this are the analyses of waste waters, industrial streams or crops and foodstuffs. For such samples, we recommend concentration of the acrylonitrile and its separation from other components by azeotropic distillation with *iso*propyl alcohol, the procedure being similar to that of Daues and Hamner,[2] who distilled azeotropically with methanol and determined acrylonitrile in the distillate by polarography. When only small amounts of sample are available, *e.g.*, from physiological material, such as urine and blood, we prefer to separate acrylonitrile by distillation in air in any apparatus generally used for analysing volatile substances.

The only polar solvents with which acrylonitrile forms azeotropic mixtures that boil at a comparatively low temperature are methanol and *iso*propyl alcohol. The azeotrope with methanol, which contains 38·7 per cent. by weight of acrylonitrile and boils at 61·4° C,[3] was used by Daues and Hamner. This azeotrope, however, is unsuitable for paper chromatography, as, under the conditions of our method, methanol and thiourea form an *iso*thiuronium spot (R_F 0·28) similar to that formed by acrylonitrile and thiourea (R_F 0·25); this causes an apparent "tailing" of the acrylonitrile spot. *iso*Propyl alcohol, on the other hand, forms a suitable azeotrope, which contains about 56 per cent. by weight of acrylonitrile and boils at 71·7° C.[4] It forms no *iso*thiuronium spot with thiourea under the conditions of the test. Another advantage of *iso*propyl alcohol for the separation of acrylonitrile is the ease with which it can be "salted out" from aqueous solution by adding sodium chloride.

The distillation apparatus consists of a 1-litre flask, the neck of which is connected to a 3·5-cm diameter glass column, 40 cm in length; this column is connected to a 2-cm diameter glass column, also 40 cm in length. Both columns are packed with glass beads, 0·5 cm in diameter, and the bottom of the lower column contains a glass-wool plug. The upper column is connected by means of a distillation head to a Liebig condenser, 21 cm in length. The distillate is collected, by way of a connecting tube, in a 20-ml flask surrounded by ice. The temperature is measured by a thermometer in the distillation head.

METHOD

REAGENTS—

iso*Propyl alcohol*—Analytical-reagent grade.
Sulphuric acid, concentrated—Analytical-reagent grade.
Sodium chloride—Commercial grade.
Thiourea—Analytical-reagent grade.
Hydrochloric acid, concentrated—Analytical-reagent grade.

PROCEDURE—

Measure a 500-ml sample of water or an aqueous suspension of about 250 g of foodstuffs, grains, etc., containing 0·5 to 100 mg of acrylonitrile, into the distillation flask. Add 25 ml of *iso*propyl alcohol and 5 ml of concentrated sulphuric acid, and mix the solution thoroughly. Saturate the solution with sodium chloride (180 g), add a few pieces of porous porcelain and glass, and attach the flask to the distillation apparatus. Place 0·1 g of thiourea and 0·15 ml of concentrated hydrochloric acid in the receiver if 2 ml of distillate are to be collected, or proportionately more for a larger volume of distillate. Heat the flask gently, so that distillation proceeds as slowly as possible and takes 45 to 60 minutes. For an acrylonitrile content of 0·5 to 20 mg, collect 2 ml of the azeotrope, but, for higher acrylonitrile contents, collect 5 to 10 ml. Distillation takes place at approximately 78° C. Treat the distillate by the method previously described.[1]

*iso*Propyl alcohol recovered by rectification from these procedures must not be used for the azeotropic distillation.

It is worth noting that crops sprayed with commercial acrylonitrile pesticides, which contain hydrocyanic acid and lactonitrile, easily absorb hydrocyanic acid by addition to carbohydrates and proteins. The bond is durable, and hydrocyanic acid cannot be removed by milling the grains or even by boiling and baking the flour products.

We thank M.U. Dr. K. Symon, University Docent, Director of The Institute of Hygiene, Prague, for his kind permission to publish this Note.

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THE DETERMINATION OF TETRABROMOETHANE AND MINERAL ACIDS DISSOLVED THEREIN

Heavy liquids are being investigated in our laboratories for the beneficiation of ores. In conjunction with these studies, a rapid method was required for determining tetrabromoethane. A suitable assay procedure for this compound is based on the ease with which it undergoes dehydrohalogenation in alkali.[1] The reaction is as follows—

$$CHBr_2{-}CHBr_2 + OH^- \rightarrow CHBr{=}CBr_2 + Br^- + H_2O$$

Reaction occurs in an alcoholic solution even at 0° C with 0·1 *N* sodium hydroxide, or on warming with sodium acetate in alcohol. Direct titration of a hot alcoholic solution of the compound with dilute alkali is sufficient to effect quantitative dehydrohalogenation. The error of the determination is within that of the burette.

It must be pointed out that the procedure is not specific for tetrabromoethane, as other compounds react in a similar manner.

The results must be corrected for the presence of hydrobromic acid, a decomposition product that may be formed if tetrabromoethane is set aside for any length of time. Determination of the acid in the sample is complicated by the susceptibility of the polyhaloalkane to dehydrohalogenation. A suitable reagent for the purpose is a mixture of potassium iodate and potassium iodide, which reacts with acid according to the following equation—

$$KIO_3 + 5KI + 6H^+ \rightarrow 6K^+ + 3H_2O + 3I_2$$

Method

Procedure for determining amounts of tetrabromoethane greater than 150 mg—

Weigh the sample into a 100-ml Erlenmeyer flask. Add 20 to 30 ml of neutral methanol, and heat to the boiling-point. Add 5 to 10 drops of phenolphthalein indicator solution, and titrate rapidly with 0·1 *N* sodium hydroxide. When a red colour appears, boil the solution again. Should the colour fade, add more 0·1 *N* sodium hydroxide, and repeat the procedure until the colour remains after the solution has been boiled for 10 seconds; this is the end-point.

This determination can be checked by an argentimetric titration on the resulting solution. Add 0·1 *N* acetic acid dropwise until the residual colour has been discharged, and then titrate with 0·1 *N* silver nitrate. Use 10 drops of a 0·01 per cent. solution of dichlorofluorescein as indicator. The total volume of solution used for the bromide determination should be less than 50 ml.

$$\text{Dehydrohalogenation equivalent} = \frac{\text{Weight of sample, g} \times 10{,}000}{\text{0·1 } N \text{ sodium hydroxide titre, ml}}.$$

For the pure compound, the dehydrohalogenation equivalent is equal to the molecular weight.

Procedure for determining amounts of tetrabromoethane between 2 and 150 mg—

Proceed as described for amounts greater than 150 mg, but use 0·01 *N* reagents. The sample must first be titrated to the red colour of phenolphthalein so that an excess of alkali is present to ensure complete dehydrohalogenation. However, as the end point of the alkali titration is not sufficiently sharp, only the subsequent bromide determination can be applied. Neutralise the solution by dropwise addition of 0·01 *N* acetic acid, and then titrate with 0·01 *N* silver nitrate.

$$\text{Dehydrohalogenation equivalent} = \frac{\text{Weight of sample, g} \times 100{,}000}{\text{0·01 } N \text{ silver nitrate titre, ml}}.$$

The minimum concentration of tetrabromoethane that can be determined by this procedure is 100 mg per litre of solvent (*e.g.*, *iso*propyl alcohol or kerosine).

PROCEDURE FOR DETERMINING ACID IN TETRABROMOETHANE—

Add starch indicator solution and an excess of aqueous potassium iodate and potassium iodide to a solution of the sample in methanol. Titrate the liberated iodine with 0·1 *N* sodium thiosulphate.

These procedures may also be applicable to other polyhalogenated alkanes.

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Received *July 10th*, 1958

A RAPID MICRO METHOD FOR DETERMINING CARBON TETRACHLORIDE IN BLOOD

PUBLISHED methods for determining carbon tetrachloride in blood involve either steam distillation or aeration to isolate the material. They are not applicable to rapid serial determinations or for determining low levels of carbon tetrachloride.[1]

By using acetone to precipitate serum proteins and to extract carbon tetrachloride simultaneously, we have found that carbon tetrachloride can be determined in the filtrate by direct application of the Fujiwara reaction.[2]

The recovery of carbon tetrachloride added to blood has been 95 per cent.; the lowest limit of sensitivity is 4 μg per ml of blood.

METHOD

REAGENTS—

Sodium hydroxide solution, 20 per cent. w/v—This solution should be freshly prepared from analytical-reagent grade material in distilled water.[3]

Pyridine—This should be colourless and of the highest purity obtainable. If it is yellowish or contains any halogenated organic compounds that give the colour reaction, redistil at a pressure of 760 mm of mercury, and use the fraction that distils at 115° C.

Acetone—Analytical-reagent grade. This must not contain any halogenated organic compounds or more than 2 per cent. of water.

PROCEDURE—

To avoid loss by diffusion into the air, take sufficient heparinised blood almost to fill a container. Analyse the sample as soon as possible (results were lower by 6 to 8 per cent. when samples were stored overnight in a refrigerator). Place a 3-ml portion of blood in a 15-ml stoppered centrifuge tube, add 7 ml of acetone, and shake. Replace the stopper, set the tube aside for 3 to 4 minutes, shake again, and spin in a centrifuge for 5 minutes at between 2000 and 2500 r.p.m. and approximately 12-cm radius.

By pipette, place 7 ml of pyridine in a 6-inch × $\frac{5}{8}$-inch thin-walled test-tube, add 2 ml of the clear supernatant liquid, and mix. (If the test-tube is not thin-walled, the heating period of 2½ minutes is insufficient.) Add 3·5 ml of 20 per cent. w/v sodium hydroxide solution, and shake the test-tube gently but thoroughly to bring the two layers into contact.

Cover the test-tube with a $\frac{15}{16}$-inch diameter glass marble to serve as a condenser and minimise losses by evaporation. Heat the test-tube to 100° C in a bath of boiling water for exactly 2½ minutes (note that these are optimum conditions; if the boiling-point of water is decreased by altitude, increase it by adding glycerol). Cool the test-tube thoroughly in tap-water for 2 minutes, and immediately transfer 5 ml of the coloured pyridine layer to another test-tube, add 1·5 ml of absolute ethanol to remove turbidity, and shake. (Continued contact between the pyridine and alkali layers causes fading; with immediate and careful separation of the pyridine layer,[4] the colour is stable for at least 3 hours.)

Measure the colour intensity at 535 mμ with a photometer. Use blood from a normal animal as blank, and read the concentration of carbon tetrachloride from an appropriate calibration graph. (The calibration graph is linear up to a concentration of 175 μg of carbon tetrachloride per ml; at higher concentrations, dilution with absolute ethanol is necessary.)

A standard carbon tetrachloride - blood solution having approximately the same concentration as the sample should be analysed simultaneously with each batch, especially at extremely low concentrations of carbon tetrachloride.

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3. Webb, F. T., Kay, K. K., and Nickol, W. E., *J. Ind. Hyg.*, 1945, **27**, 249.
4. Fabre, R., Truhaut, R., and Laham, S., *Ann. Pharm. Franç.*, 1951, **9**, 251.

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Apparatus

MODIFICATION TO THE UNICAM SP500 SPECTROPHOTOMETER FOR SINGLE-BEAM RECORDING

(*Presented at the meeting of the Society on Wednesday, November 5th*, 1958)

ALL the ultra-violet spectrometric measurements carried out in these laboratories are made with a Unicam SP500 spectrophotometer. Our work includes both quantitative and qualitative measurements on samples in solution, and is almost entirely limited to the wavelength range between 2200 and 3000 A. Past experience has shown that the plotting of a spectrum for qualitative purposes involves a considerable amount of work and time, which is out of proportion to the information obtained and leads to a disinclination to use the technique. It was decided, therefore, to look for a simple and inexpensive method whereby the spectra could be plotted more quickly and easily, preferably by using an automatic recording device.

It was necessary that, in any modification to the instrument itself, it should still be possible to use the instrument in its conventional manner for quantitative measurements. The modification described by Kendall and Lloyd[1] was considered to be of greater complexity than was desired to meet our requirements, especially since the spectra were intended for qualitative purposes only. Beckman Instruments Inc. brought out an energy recording attachment for their DU spectrophotometer, and the modified circuit has been described by Cahn.[2] Since the Unicam SP500 and the Beckman DU spectrophotometers are basically similar, this modification can easily be adapted to the Unicam instrument. It has the merit of simplicity, as it requires only two extra resistors, a potentiometer and an additional 1½-volt supply to permit the output from the amplifier in the instrument to be put on to a potentiometer pen recorder. A multi-pole two-way switch is used to select either the recording or the conventional null-balance circuit.

DESCRIPTION OF THE APPARATUS—

In our modification, it was found convenient to house all the extra electrical components in a small brass box mounted on the back of the photocell box. The normal filament supply for current valve (red label) cannot be used in the recording modification, so an alternative supply was obtained by tapping off 2 volts from the accumulator used for lighting the tungsten lamp and using it to supply the filament through a dropping resistor and the conversion switch.

The output of this single-beam energy recorder when the wavelength knob is scanned will be a combined plot of the output of the lamp, the transmission of the instrument and sample and the sensitivity of the photocell at each wavelength. With no sample in the beam and with a fixed slit width, this output will be far from constant and will be of limited use. Normally, with single-beam spectrophotometers, the slit width is constantly altered to provide a constant background energy, and this problem has been simply solved by Kendall and Lloyd,[1] who obtained satisfactory background curves by rotating the slit knob at a rate directly proportional to that of the wavelength knob. Experiments confirmed that this was also true for our instrument over the range approximately 2200 to 3300 A with the slit knob being driven at four-thirds the speed of the wavelength knob, and a slit width of about 0·5 to 0·6 mm at 2400 A.

Since the wavelength scale of the instrument is not linear with angular rotation of the wavelength knob, it was decided to drive this knob at a non-linear rate, the aim being to scan equal wavelength intervals in as nearly equal intervals of time as possible.

The drive is applied to the wavelength and slit-control shafts of the instrument through Meccano sprocket chain and sprocket wheels, the latter being mounted behind the two knobs

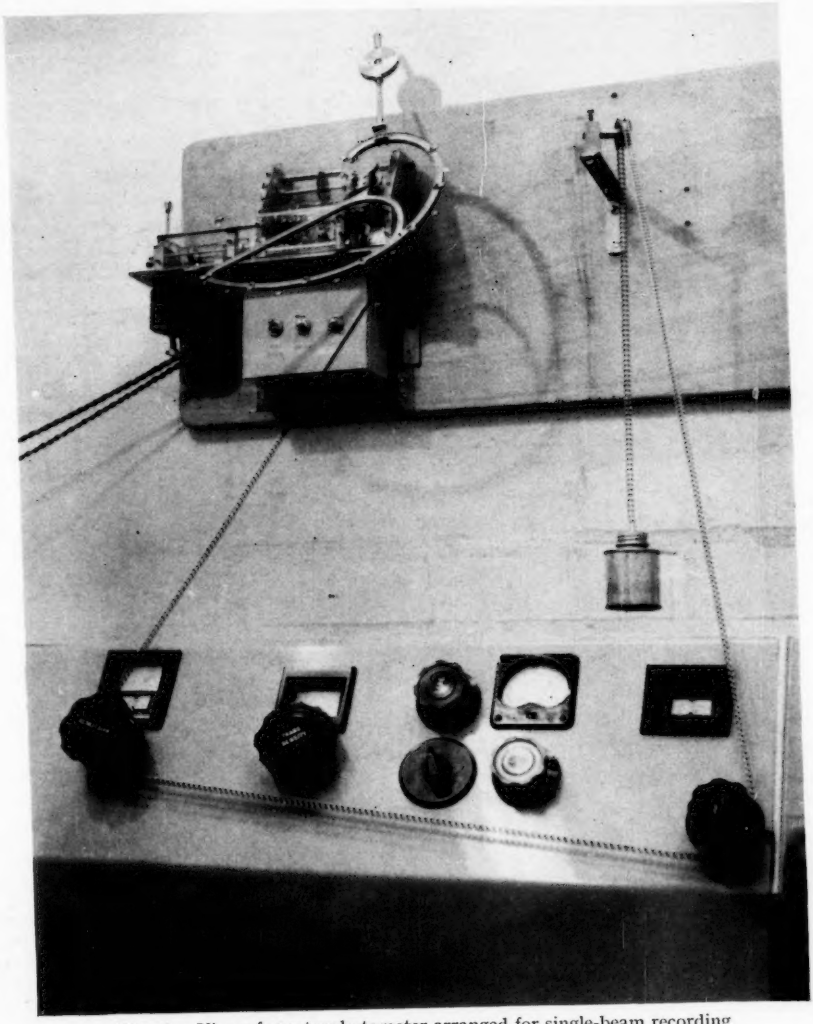

Fig. 1. View of spectrophotometer arranged for single-beam recording

[To face p. 68.

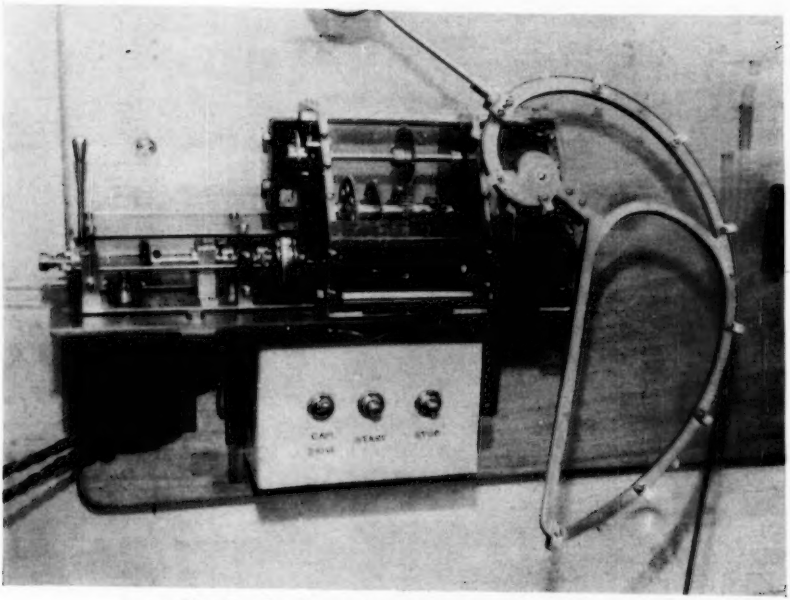

Fig. 2. Gearbox and wavelength-cam drive unit

in question. A 2-inch diameter sprocket wheel on the wavelength drive was adopted as a convenient size. The actual driving force is a weight (approximately 600 g) and one end of the chain (at the slit-control end of the instrument) is attached to it after passing over a pulley mounted on the wall above and behind the instrument. The other end of the chain is attached to a length of steel tape, which is wrapped round the circumference of a cam and is attached to the point of its circumference nearest its axis of rotation (see Fig. 1).

The shape and size of the cam is calculated to turn the wavelength scale over the required wavelength range in one rotation of the cam spindle. Its form can be seen from Figs. 1 and 2, and its circumference forms the part of the curve whose polar equation is—

$$\log_e r = 0{\cdot}391\ \theta - 0{\cdot}4805$$

for values of θ from 0 to 2π, where r is the radius vector in inches and θ is measured in radians, the axis of rotation of the cam being the origin of the polar curve. The resulting wavelength scale is nearly linear over the range in question.

The cam spindle is meshed through Meccano gears to a Venner synchronous motor with a shaft speed of 1 revolution in 6 minutes. The gear-box (see Fig. 2) will allow the selection of 1 cam rotation in 18, 10, 6 or 3·6 minutes, but, for various reasons, the 10-minute scan is the most often used. An additional shaft, with a speed always $1\frac{1}{4}$ times that of the cam spindle, is provided with a micro-switch contact, which, via a relay, stops the motor at the end of the scan, *i.e.*, 3280 A, and also stops the chart-drive motor of the pen recorder. This is to enable the operator to leave the instrument running while the next sample is being prepared, without fear of the cam and its tape over-winding. A dog-clutch mechanism is inserted between the motor and the gear-box. By disengaging the clutch while supporting the cam with the hand, the cam can be rotated backwards to the start for the next run; the clutch is then re-engaged. This start is usually at 2200 A, although, of course, any wavelength may be chosen to suit the requirements of the test.

The drive controls are such that the scanning and the chart-drive motors may be started and stopped simultaneously by means of push-buttons, and separate switches are provided so that either the recorder or scanning system may operate independently. This makes it possible to operate the recording device at a fixed wavelength.

The recorder used is an Ether wide-strip potentiometer recorder with 5-second response and 50-mV full-scale deflection. A chart speed of 1 inch per minute is normally used, although other speeds are available for different scales. The recorder has manual standardisation, so that uninterrupted spectra may be obtained.

PROCEDURE FOR PLOTTING A SPECTRUM—

The instrument is easily set up for a recording run. The cam is rotated forwards to the point at which the micro-switch operates. The wavelength knob is then set to an arbitrarily chosen wavelength (3280 A for our work) and the chain is engaged on the wavelength-sprocket wheel. The slit width is similarly set up to a standard value at any wavelength. The change-over switch is turned to the RECORD position. With a cell containing solvent in the beam, the cam is rotated by hand and the output control is adjusted so that the trace will all be within the range of the recorder. The solvent spectrum and then the spectra of the samples can be scanned, the output-control setting being the same in each instance.

The shape of the background curve can be adjusted to allow for the effects of the deterioration of the hydrogen lamp with time by altering the slit width at the setting up point. A little more drastic change may be brought about by changing the diameter of the sprocket wheel on the slit-drive shaft.

Although this modification was not expected to give an accurate measure of the transmission of the sample, it has, in fact, proved to be accurate over the whole scale to within a maximum error of 1 per cent. transmission. The linearity is affected considerably by variation of the voltage applied to the heater of the current valve, and also by changing the tappings on the dry batteries, B1 and B2. Variations in B4 also affect the linearity, but B3 has no effect on this function (over small changes). It has been found possible to adjust the various battery voltages to values that give a satisfactory performance when the instrument is used in the conventional manner, and that, at the same time, give an almost linear output on the recorder.

As an illustration of the performance of the instrument, reproductions of the recorded spectra of *cyclo*hexane and solutions of benzene and diphenylene oxide in *cyclo*hexane are shown in Figs. 3 and 4.

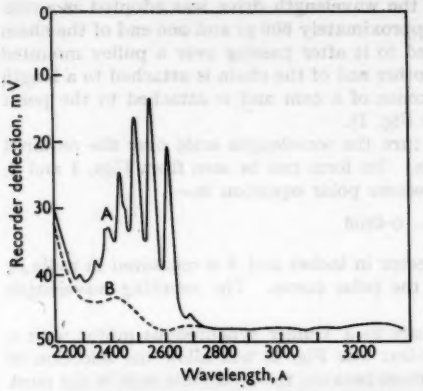

Fig. 3. Spectrum in 1-mm cell of benzene in *cyclo*hexane: curve A, benzene; curve B, background (*cyclo*hexane)

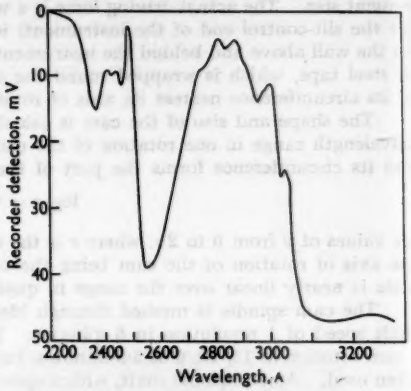

Fig. 4. Spectrum in 1-mm cell of diphenylene oxide in *cyclo*hexane

Recently, the range over which the recording instrument can operate has been extended to 4000 A. The tungsten lamp is, of course, used over this additional range, and it has been found necessary to use a smaller sprocket wheel (¾ inch) on the slit drive to obtain the best results. The background (solvent) curve is not as flat as could be hoped for, but is sufficiently satisfactory for the purpose of sample identification. The 1½-inch and ¾-inch sprocket wheels are now both mounted on the slit-drive shaft behind the knob.

I thank the Directors of the Midland Tar Distillers Ltd. for permission to publish this work, and Miss C. Wale for her assistance.

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THE MIDLAND TAR DISTILLERS LIMITED
RESEARCH DEPARTMENT
FOUR ASHES
NR. WOLVERHAMPTON

D. D. SHREWSBURY
Received *June 9th*, 1958

Discussion

DR. J. HASLAM asked how the instrument was used in liquid - liquid partition chromatography.

MR. SHREWSBURY replied that the question was related to another distinct programme of work not carried out by himself. The spectrophotometer was adjusted to a predetermined fixed wavelength and would detect and record materials separated by a liquid - liquid partition chromatographic column. This normally entailed leaving the instrument running overnight, which meant that they could get 24 hours' use from the Unicam SP500 spectrophotometer each day.

DR. G. E. FOSTER enquired whether the recording device gave reproducible results and whether the scanning time was constant.

MR. SHREWSBURY said that they had been completely satisfied by the stability and reproducibility of the instrument as a whole. The dark current would normally drift by only 2 or 3 per cent. on overnight runs and the emission of the hydrogen lamp was extremely constant, unless it was near the end of its useful life, when it could produce spikes on the trace.

The drive unit had then been in use for over a year with only minor troubles. The instrument was its own fault-finder, for if the length of the recorded spectrum was not correct then the drive unit was not functioning properly.

Book Reviews

BIOLOGICAL TREATMENT OF SEWAGE AND INDUSTRIAL WASTES. Edited by Brother JOSEPH McCABE, F.S.C., and W. W. ECKENFELDER, JUN. Volume II. Anaerobic Digestion and Solids-Liquid Separation. Pp. vi + 330. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1958. Price $10.00; 92s.

This book is a collection of twenty-eight papers, many of them under joint authorship, presented in April, 1957, to a conference at Manhattan College, New York, on aspects of biological treatment of sewage and industrial wastes. The volume is the second in a series and is divided into three parts devoted to anaerobic digestion, sedimentation and flotation, and vacuum filtration and sludge conditioning.

The convenience of having available, in one volume, up-to-date accounts of laboratory and pilot-plant investigations into highly specialised problems is beyond dispute, and anyone who is more than casually concerned with the subjects of separation, stability and disposal of biologically active sludges and is already acquainted with the elementary principles of these subjects, will find the book well worth careful study. It contains information that has much practical value for sewage works chemists, sanitary engineers and designers of treatment plant, and if, on balance, some of the experiments reveal more problems than they solve, that is the inevitable consequence of scientific penetration into subjects that hitherto have been mainly tackled empirically. Those who have facilities for research into the problems of large-scale waste disposal cannot fail to be stimulated by this collection of papers, many of which have the additional advantage of being interim accounts of work that is not yet completed.

The printing and presentation of the book are good; the many diagrams are clear and easily understood and proof reading has been most conscientious and thorough. J. G. SHERRATT

RADIOAKTIVE ISOTOPE IN DER BIOCHEMIE. By ENGELBERT BRODA. Pp. viii + 326. Vienna: Verlag Franz Deuticke. 1958. Price Austrian S294.

This book by Professor Broda of the University of Vienna is the first to come from central Europe on the applications of radioactive isotopes in biochemistry; it is of special interest to English readers on this account, since it includes references to recent continental and Russian papers that are sometimes overlooked.

Professor Broda's monograph is exceptionally well documented and includes literature references to the end of 1957. This, in view of normal publishing delays, is a remarkable achievement.

The first one-third of the book is devoted to a review of the principles and techniques of radioactive-isotope procedures, the methods of measuring radioactivity and the use of radioactive isotopes for the analysis of natural substances. The remainder of the book describes how isotopes have been, and are being, used to shed light on and to solve the wide range of fundamental biochemical problems that cannot be tackled by other analytical techniques.

The book is well printed, but does not have a stiff cover. J. E. PAGE

Publications Received

SYMPOSIUM ON NUCLEAR SEX. Edited by D. ROBERTSON SMITH, M.A., M.D., and WILLIAM M. DAVIDSON, M.D. Pp. xviii + 188. London: William Heinemann Medical Books Ltd. 1958. Price 21s.

BIOLOGICAL METHODS FOR THE EVALUATION OF RODENTICIDES. By E. W. BENTLEY, B.Sc., Ph.D. *Technical Bulletin No.* 8. Pp. iv + 35. London: Her Majesty's Stationery Office. 1958. Price 3s.

BIBLIOGRAPHY ON MOLTEN SALTS. Compiled by GEORGE J. JANZ. Pp. viii + 73. New York: Rensselaer Polytechnic Institute. 1958. Price $1.00.

EDTA: A BIBLIOGRAPHY OF ANALYTICAL USES. Pp. ii + 73. Manchester: The Geigy Company Ltd. 1958.

XV CONGRESSO INTERNACIONAL DE QUIMICA PURA E APLICADA (*Quimica Analitica*): Actas do Congresso (*8 a 16 de Setembro de* 1956). Volume I. Pp. 1028. Lisboa: General Secretary of the XVth International Congress of Pure and Applied Chemistry, Instituto Superior Technico. 1957. Price, together with volumes II and III, 320 Escudos.

Volumes II and III will be published shortly.

CHOLESTEROL. By DAVID KRITCHEVSKY. Pp. xii + 291. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1958. Price $9.75; 78s.

OUTLINES OF ENZYME CHEMISTRY. By J. B. NEILANDS and PAUL K. STUMPF. Second Edition. Pp. xii + 411. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1958. Price $8.50; 68s.

HANDBOOK OF CHEMICAL MICROSCOPY. Volume I. By EMILE MONNIN CHAMOT, B.S., Ph.D., and CLYDE WALTER MASON, A.B., Ph.D. Third Edition. Pp. xii + 502. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1958. Price $14.00; 112s.

GAS CHROMATOGRAPHY 1958. Edited by D. H. DESTY. Pp. xiv + 383. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1958. Price 70s.; $12.00.

Proceedings of the second symposium organised by the Gas Chromatography Discussion Group under the auspices of the Hydrocarbon Research Group of the Institute of Petroleum and the Koninklijke Nederlandse Chemische Vereniginghеld at the Royal Tropical Institute, Amsterdam, 19–23 May, 1958.

REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY. Volume XLII: 1957. Editor: H. S. ROOKE, M.Sc., F.R.I.C. Pp. 910. London: The Society of Chemical Industry. 1958. Price 100s.

Papers for Publication in *The Analyst*

THE Editor welcomes Papers and Notes for insertion in *The Analyst*, whether from members of the Society or non-members. They are submitted to the Publication Committee, who decide on their suitability for insertion or otherwise.

A copy of the current Notice to Authors, last published in full in *The Analyst*, 1957, **82**, 719, can be obtained on application to the Editor, *The Analyst*, 14 Belgrave Square, London, S.W.1. All Papers submitted will be expected to conform to the recommendations there laid down and any that do not may be returned for amendment.

Errata

NOVEMBER (1957) ISSUE, p. 765, 10th line from foot of page. *For* "1 ml ≡ 100 mg of barium" *read* "1 ml ≡ 100 μg of barium."

The corresponding correction should be made to "Recommended Methods for the Analysis of Trade Effluents" (Cambridge: W. Heffer & Sons Ltd., for the Society for Analytical Chemistry, 1958) at p. 54, 5th line.

MAY (1958) ISSUE, p. 285, 14th line. *For* "$E_P = E_P = 0$" *read* "${}_AE_P = {}_BE_P = 0$."

IBID., p. 287, 1st and 2nd lines under Table II. *For* "between 92·1 and 95·7 per cent. The mean value, 93·7 per cent., is the same as that reported by Ketelaar" *read* "between 92·1 and 95·7 per cent., the mean value being 93·7 per cent."